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# DETOXICATION MECHANISMS

## *The Metabolism of Drugs and Allied Organic Compounds*

□

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# *Preface*

THE last ten to fifteen years have seen a rapid increase in the number of synthetic organic compounds used in the treatment of disease. What happens to such organic compounds in the body therefore becomes an important question. We cannot, as yet, predict accurately what would be the fate of a substance of known structure. There is, however, sufficient information to allow us to make a rough guess. In writing this book, my object has been to gather together in orderly fashion the available information on the metabolic fate of organic compounds foreign to the body, so that working hypotheses can be advanced. In the past the subject has been treated very briefly in textbooks of Biochemistry under the title of "Detoxication" or "Detoxication Mechanisms", and I have adopted the latter term as the short title for this book.

Our knowledge of the metabolic fate of even simple organic molecules is often incomplete, for in most cases a balance sheet has not been worked out. It may take several years of investigation before the fate in the body of an individual substance is fully established. In the past most of the work in this field has been qualitative. It is therefore important to draw attention to frequent statements in the literature to the effect that a compound A is transformed in the body to a compound B without reference to the quantitative aspects. In such cases it is probable that other metabolites are formed and have not yet been detected.

The results of investigations into detoxication mechanisms can affect many branches of chemical and medical research and must in time influence teaching. This book is intended to guide various types of readers as well as the specialist, and I have tried to deal with the subject in a manner capable of assisting a wider audience than the experts in the field.

Although it is hoped that little relevant material published before 1945 has been missed, the years 1945 and 1946 have not been covered fully. This is an inevitable result of delays arising out of the War.

During the writing and the preparation of this book for the press I have received valuable help and suggestions from my colleagues, Professor R. A. Morton, Mr. T. W. Goodwin and Dr. A. L. Stubbs. Professor Morton read the typescript and suggested many improvements, Mr. Goodwin read and corrected the typescript and proofs at all stages, and Dr. Stubbs read the galley proofs. It is therefore a great pleasure to express my feelings of gratitude to these three, for they have contributed greatly to the clearness and accuracy this book may possess. It is also a pleasure to acknowledge my indebtedness to Miss B. M. Morris for typing the manuscript.

R. T. W.

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## *Chapter One*

# Introductory and Historical

IT is undoubtedly true that the field of study which is conveniently named " detoxication " has now broadened to include all those metabolic processes not specifically covered by the main streams of fat, carbohydrate and protein intermediary metabolism. Detoxication is, in fact, the study of the metabolism of organic compounds other than lipides, carbohydrates, proteins and closely related natural compounds, although the line of demarcation between these two groups is by no means a sharp one. Thus the metabolic changes undergone by benzene when administered to animals would be designated as " detoxication mechanisms " (or " detoxication reactions or processes "), whereas those undergone by phenylalanine would not. One could say that all compounds such as benzene which are not normally utilised by the body and do not occur to any large extent in food materials are toxic in the sense that they are foreign to the body, although they may not be intrinsically toxic.

Although the term " detoxication " or " detoxication mechanism " implies some process or processes whereby toxicity in a compound is reduced or abolished, in its modern usage it includes not only changes involving a reduction in toxicity but also those involving an increase in toxicity. If, therefore, the term is taken in its literal sense it becomes in many cases a misnomer. Nevertheless, it is a firmly established term in biochemistry and provides, if properly defined, an acceptable designation for a broad field of study. However, it may be desirable in order to avoid ambiguity to introduce more specific terms possessing precise and literal meaning. This could be achieved by subdividing detoxication mechanisms into hypertoxic, isotoxic and hypotoxic changes when and where it would be advantageous to do so. It should, however, be noted that the primary objectives of most workers in this field are to elucidate catabolic processes and that in many problems the relative or absolute toxicities of metabolites may not be of immediate importance. Consequently one finds that few attempts have been made to measure toxicities in such studies. This fact does not imply that toxicity is of minor significance but that it constitutes only one aspect, albeit an important one, of detoxication mechanisms, and one which should merit more attention in the future. Detoxication need therefore mean no more and no less than the qualitative and quantitative study of the metabolism of foreign organic compounds, and if it is desired to use the word detoxication in its literal sense, the expression " true detoxication " (cf. " haemoglobin " and " reduced haemoglobin ") might satisfy the pharmacologist when reduction in toxicity actually occurs and " pseudo-detoxication " in other cases.

The standpoint of this book is simply that the term detoxication (or detoxication mechanisms) means those chemical changes which foreign organic compounds undergo in the animal body. This view is consistent with that of most modern workers in the field ; Young (1939), for example, states : " It has become customary to describe as ' detoxication reactions ' those chemical changes occurring in the body which bring about the conversion of foreign compounds of known structure to derivatives which are excreted in the urine " (cf. also Stekol, 1941).

## The Biochemical Changes known as Detoxication Mechanisms

Foreign organic compounds, when administered to animals, may undergo a number of definite chemical changes, resulting in the excretion *via* the kidney of specific metabolites. The type of change which may occur is dependent primarily upon the chemical structure of the compound administered, but in some cases the kind of animal under observation may be of great importance, for a given compound may follow totally different metabolic routes in different species. The original compound may also be excreted unchanged, wholly or in part.

The more usual changes or detoxication reactions are oxidative, reductive or synthetic in nature. The *oxidations* and *reductions* are many and varied, but in general, compounds of similar structure are oxidised or reduced *in vivo* in a qualitatively similar manner. The *synthetic reactions*, or conjugation processes, as they are often called, are limited in number, and so far only nine have been established. They are :—

(1) the glucuronic acid conjugation,
(2) the ethereal sulphate conjugation,
(3) the glycine conjugation or hippuric acid synthesis,
(4) the cysteine conjugation or mercapturic acid synthesis,
(5) the ornithine conjugation or ornithuric acid synthesis,
(6) the glutamine conjugation,
(7) acetylation,
(8) methylation,
(9) the thiocyanate detoxication.

The occurrence of a particular conjugation process depends upon the nature of the compound administered and upon the species of animal under observation. A given compound will not undergo any of these syntheses unless it possesses an appropriate structure as a whole or carries the necessary group or groups for conjugation. If the compound does not carry a group necessary for conjugation it may acquire such a group either by oxidation or reduction *in vivo*. Many cases are in fact known of oxidation or reduction preceding a conjugation. With regard to different species of animals, it has been found that some conjugation processes are confined to one or two species, whilst others tend to occur in all or most species. Thus, the glutamine conjugation has only been observed in man and the chimpanzee, whilst the ornithine conjugation occurs only in birds. On the other hand, the glucuronic acid and ethereal sulphate conjugations have been found to occur in all species studied, and the glycine conjugation, the cysteine conjugation, acetylation and methylation occur in most species. There are exceptions in that the rabbit cannot methylate, the pig does not form mercapturic acids, the bird does not use glycine and the dog cannot acetylate the aromatic amino group.

A further point to be noted about the synthetic mechanisms is that part of the molecule synthesised is provided by the organism. This moiety may be referred to as the conjugating agent. When the dose of the foreign compound is not excessive, the conjugating agent may be provided from waste material or from the tissues, without undue strain upon the resources of the animal. With excessive doses, however, the conjugating agent may be utilised for detoxication at the expense of material required for the well-being of the organism. This is well illustrated by the case of bromobenzene which, if fed to growing animals in excess of a certain dose, causes cessation of growth because the animal is unable to provide sufficient cysteine for both growth and the detoxication of the excessive amounts of bromobenzene. Here, apparently, the detoxication process takes precedence over growth.

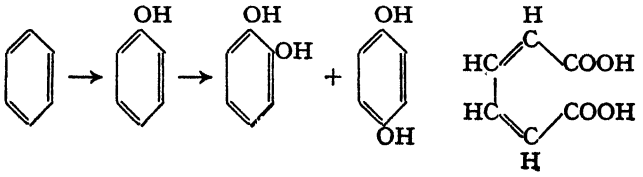
A brief account will now be given of individual detoxication mechanisms. These represent the main pathways of the metabolism of foreign organic compounds in the animal body.

## OXIDATION

A large variety of foreign organic compounds undergo oxidation in the animal body, and the mode of oxidation is largely dependent upon the structure of the compound. A discussion of the oxidations which may occur in the animal body is out of place in this book since it would cover almost the whole subject of biochemistry. At this point it will be sufficient to indicate one or two general pathways of oxidation which have been classified as detoxication reactions.

Many primary alcohols, aliphatic and aromatic, are oxidised *in vivo* to the corresponding acids and it is likely that an aldehyde is an intermediate in this type of oxidation. Thus methyl alcohol and benzyl alcohol are oxidised in the animal body to formic acid and benzoic acid respectively.

Aromatic hydrocarbons, whether monocyclic or polycyclic, are in many cases oxidised to phenols. Oxidative opening of the aromatic ring is rare, and when it happens occurs only to a slight extent. A typical example is that of benzene, which in the animal is oxidised partly to phenol, quinol and catechol, and in a minor degree to the unsaturated muconic acid.

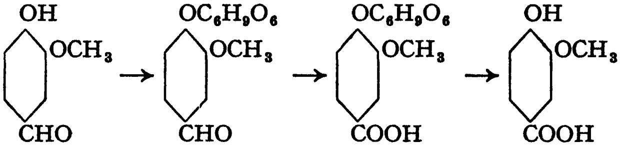


The order in which various oxidation products of benzene appear is not clear.

Methyl groups are largely oxidised to carboxyl groups, primary alcohols and aldehydes being intermediates thus :—

$$—CH_3 \rightarrow —CH_2OH \rightarrow —CHO \rightarrow —COOH$$

Oxidation may often be followed by a synthetic reaction, thus phenol, formed during the bio-oxidation of benzene, may be combined in the body with sulphuric or glucuronic acid. Alternatively, a synthetic reaction may precede oxidation, since it appears probable that vanillin may be partly combined with glucuronic acid forming glucurovanillin before it is oxidised to vanillic acid.



Many compounds, particularly those of the aliphatic series, are completely oxidised to carbon dioxide and water without detectable intermediates.

## REDUCTION

Reduction of foreign organic compounds appears not to be as common as oxidation, but for a number of chemical groups it seems to be the general biochemical reaction. The following are examples of reduction processes which have been put into the category of detoxication mechanisms. Chloral is reduced *in vivo* to trichloroethyl alcohol.

$$CCl_3CHO + 2H = CCl_3CH_2OH$$

Aromatic nitro compounds are reduced *via* hydroxylamines to amines (cf. T.N.T.).

$$ArNO_2 \rightarrow ArNHOH \rightarrow ArNH_2$$

Ketones are often reduced to secondary alcohols; thus acetophenone is reduced to methylphenylcarbinol, $C_6H_5COCH_3 \rightarrow C_6H_5CHOHCH_3$, and *l*-menthone is reduced to *d*-*neo*menthol.

In many instances biological reduction, like oxidation, appears to precede conjugation, the compound metabolized thus acquiring new groups which are necessary for the conjugation processes. Thus, trichloroethyl alcohol, which is formed by the bio-reduction of chloral, is excreted as trichloroethylglucuronide.

$$CCl_3CHO \rightarrow CCl_3CH_2OH \rightarrow CCl_3CH_2OC_6H_9O_6$$

*p*-Nitrobenzoic acid is reduced to *p*-aminobenzoic acid which is acetylated *in vivo* to *p*-acetaminobenzoic acid.

$$p\text{-}NO_2C_6H_4COOH \rightarrow NH_2C_6H_4COOH \rightarrow CH_3CO.NHC_6H_4COOH$$

## SYNTHETIC MECHANISMS

1. **Glucuronide Formation.** This is by far the most common synthetic process. If any of the nine types of reaction given on page 2 is to be regarded as a detoxication mechanism in the literal sense of the term, then certainly glucuronide formation has a strong claim. Compounds which will form conjugated glucuronic acids are those which possess or can form by oxidation or reduction in the body the following groups :

(*a*) Hydroxyl groups in almost any type of compound (except carbohydrates), e.g. alcoholic and phenolic hydroxyl groups. The hydroxyl group of hydroxylamines (—NHOH) has not, so far, been shown to form a glucuronide.

(*b*) Carboxyl groups in certain types of aromatic compounds, e.g. the —COOH attached to an aromatic nucleus as in benzoic acid, the —COOH group of phenylacetic acid and that of diphenylacetic acid (i.e. ArCOOH, $ArCH_2COOH$ and $Ar_2CHCOOH$). The aliphatic carboxyl group apparently does not form a glucuronide, except, as has been recently shown by Dziewiatkowski and Lewis (1945), when it is attached to a tertiary carbon atom. In the rabbit trimethylacetic (pivalic) and tertiary butylacetic acids are converted to a large extent into ester glucuronides :—

$$(CH_3)_3C.COOH \rightarrow (CH_3)_3C.COOC_6H_9O_6$$
$$C_2H_5(CH_3)_2C.COOH \rightarrow C_2H_5(CH_3)_2C.COOC_6H_9O_6$$

Examples of the above groups are illustrated in the following glucuronides (Table 1) which have been described and isolated.

TABLE 1

| Group conjugating | Example | Glucuronide |
|---|---|---|
| Primary alcohol<br>$R.CH_2OH$ | tribromethyl alcohol | $Br_3CCH_2O.C_6H_9O_6$ |
| Secondary alcohol<br>$R_1R_2CHOH$ | *l*-menthol<br>or<br>2-butanol | $C_9H_{18}CHO.C_6H_9O_6$<br><br>$C_2H_5CH_3CHO.C_6H_9O_6$ |
| Tertiary alcohol<br>$R_1R_2R_3COH$ | tertiary butyl alcohol | $(CH_3)_3CO.C_6H_9O_6$ |
| Phenolic hydroxyl<br>$C_6H_5OH$ | phenol | $C_6H_5O.C_6H_9O_6$ |
| Aromatic carboxyl<br>$C_6H_5COOH$ | benzoic acid | $C_6H_5CO.O.C_6H_9O_6$ |
| Phenylacetic carboxyl<br>$C_6H_5CH_2COOH$ | phenylacetic acid | $C_6H_5CH_2CO.O.C_6H_9O_6$ |
| Diphenylacetic carboxyl<br>$(C_6H_5)_2CHCOOH$ | diphenylacetic acid | $(C_6H_5)_2CHCO.O.C_6H_9O_6$ |

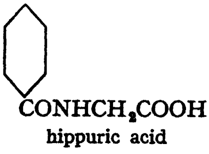
Other types of OH and COOH groups may yet be found which form conjugated glucuronides in the animal body.

The body can therefore form two types of glucuronide, namely, the ether type, e.g. phenylglucuronide and menthylglucuronide, and the ester type, e.g. benzoylglucuronide. The ether type is usually stable to alkali and non-reducing to alkaline reagents, whereas the ester glucuronides are unstable to alkali, splitting off free glucuronic acid in alkaline solution and thereby showing reducing properties when tested against the usual alkaline copper reagents for reducing sugars. An example of a combination of both types is also known for when *p*-hydroxybenzoic acid is fed to dogs, *p*-glucuronosidobenzoylglucuronide (*p*-hydroxybenzoic acid diglucuronic acid, see p. 105) is excreted. This compound contains two glucuronic acid residues attached to one hydroxybenzoic acid molecule, one by means of an ether linkage and the other by an ester linkage.

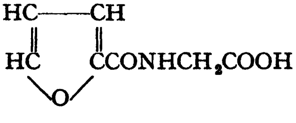
2. **The Ethereal Sulphate Conjugation.** This conjugation appears to be confined to the phenolic hydroxyl group, the product of the conjugation being an aryl sulphuric acid, R—$OSO_3H$, i.e. an acid ester of sulphuric acid or an ethereal sulphate. It is important not to confuse this group with the sulphonic acid radicle, —$SO_3H$. The ethereal sulphates occurring normally in urine are mainly those of phenol, *p*-cresol and indoxyl, *p*-cresyl hydrogen sulphate predominating. It seems likely that compounds of the purine and pyrimidine series may form ethereal sulphates since in many cases the OH groups in these series are phenolic in nature. *Iso*barbituric acid, for example (see p. **214**), when fed to dogs, causes an increase in the ethereal sulphate output. Heterocyclic phenols such as hydroxyantipyrine (see p. **229**) and hydroxyquinoline also form ethereal sulphates.

3. **The Glycine Conjugation—Hippuric Acids.** The synthesis of hippuric acid in the animal body has been known for over a century and consists of the combination of glycine with an aromatic acid forming an aroylglycine. The following types of carboxyl group are known to undergo this synthesis :—

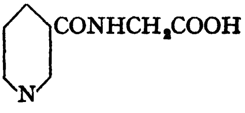
(*a*) The nuclear carboxyl groups in benzene, naphthalene, thiophene, furane or pyridine :



hippuric acid

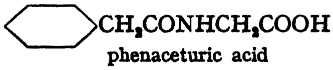


pyromucuric acid



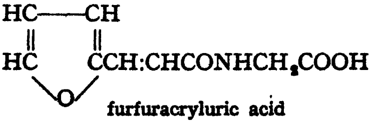
nicotinuric acid

(*b*) The carboxyl group of the phenylacetic acids :

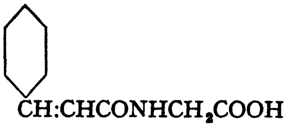


phenaceturic acid

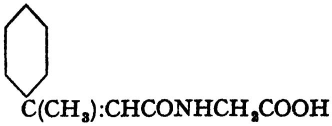
(*c*) The carboxyl group which is separated from an aromatic ring by a vinyl group, as in furfuracrylic acid and cinnamic acid. *β*-Methylcinnamic acid also forms a glycine conjugate *in vivo*.



furfuracryluric acid

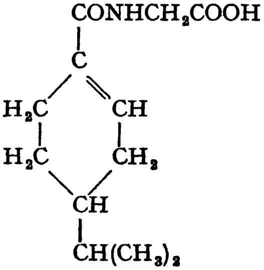


cinnamoylglycine



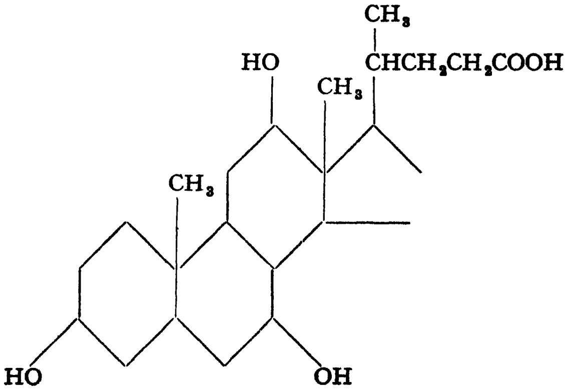
β-methylcinnamoylglycine

The possibility that carboxyl groups, attached to a tertiary carbon atom, might form glycine conjugates has been suggested by Dziewiatkowski and Lewis (1945). Such a glycine conjugate, phellanduric acid, has actually been isolated by Wright (1945) from the urine of sheep receiving *l*-α-phellandrene (see p. 170).

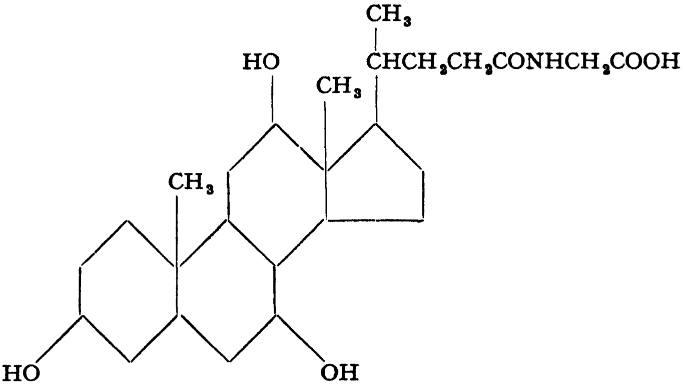


phellanduric acid

Compounds containing the aliphatic carboxyl group are not as a rule excreted combined with glycine. Human and animal biles, however, normally contain glycine conjugates, the best known of these being glycocholic acid. Desoxycholic, anthropodesoxycholic, chenodesoxycholic and ursodesoxycholic acids are also found in the biles of certain animals as conjugates with glycine (see Sobotka and Bloch, 1943). The occurrence of these conjugates suggests that the aliphatic carboxyl group in the bile acids may undergo conjugation with glycine *in vivo*. If cholic acid or desoxycholic acid is administered to human beings, there is an increase in both the free and conjugated bile acids of the bile (Doubilet, 1937) and it appears that these acids behave to some extent like aromatic acids.

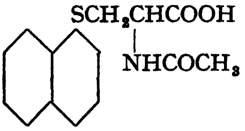


cholic acid

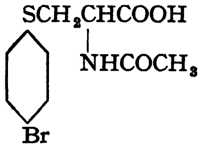


glycocholic acid

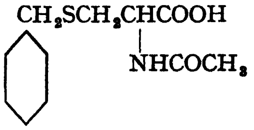
4. **The Mercapturic Acid Synthesis.** In this detoxication mechanism the amino acid cysteine acts as the detoxicating agent. During the formation of the mercapturic acid it appears that acetylation is also involved so that the part of the molecule provided by the organism is N-acetylcysteine. The types of compounds which undergo this synthesis are few and all belong to the aromatic series. These are benzene, the polycyclic hydrocarbons, halogenated hydrocarbons in which the halogen is attached to the nucleus, and benzyl chloride. In all cases except the last, the sulphur atom of cysteine is linked to a nuclear carbon atom. The following formulae indicate the types of mercapturic acid which are known :—



1-naphthylmercapturic acid (from naphthalene)

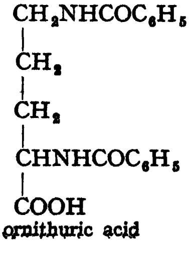


*p*-bromophenylmercapturic acid (from bromobenzene)



benzylmercapturic acid (from benzyl chloride)

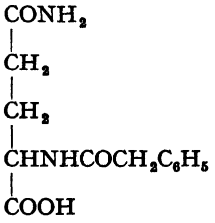
5. **The Ornithuric Acid Synthesis.** The participation of the amino acid ornithine in a detoxication mechanism is confined to birds. When benzoic acid and phenylacetic acids are fed to mammals they are in part excreted combined with glycine, but in the hen glycine is mainly replaced by ornithine, and the so-called ornithuric acids are excreted. Benzoic acid, for example, is excreted as ornithuric acid or dibenzoylornithine. *p*-Nitrophenylacetic and pyromucic acids also form ornithuric acids in the hen.



$$\begin{array}{l} CH_2NHCOC_6H_5 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CHNHCOC_6H_5 \\ | \\ COOH \end{array}$$

ornithuric acid

**6. The Glutamine Synthesis.** Whereas most mammals excrete phenylacetic acid mainly combined with glycine (e.g. the dog and the rabbit) and hens excrete it combined with ornithine, man and the chimpanzee excrete it in combination with glutamine as phenylacetylglutamine. As much as 95% of moderate doses of the acid is excreted in man combined with glutamine, the rest forming a glucuronide.



$$\begin{array}{l}CONH_2\\ |\\ CH_2\\ |\\ CH_2\\ |\\ CHNHCOCH_2C_6H_5\\ |\\ COOH\end{array}$$

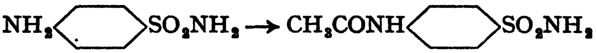
phenacetylglutamine

The glutamine is produced at the expense of waste nitrogen (see p. **109**). Substituted phenylacetic acids, such as *o*- and *p*-chloro- and *o*-bromophenylacetic acids, behave differently and form glycine, and not glutamine conjugates, in man.

$$C_6H_5CH_2COOH \rightarrow C_6H_5CH_2CONHCH(COOH)CH_2CH_2CONH_2$$
$$p\text{-}ClC_6H_4CH_2COOH \rightarrow p\text{-}ClC_6H_4CH_2CONHCH_2COOH$$

**7. Acetylation.** The only group which undergoes acetylation in the body is the amino group. There is no record that the hydroxyl group undergoes this reaction. Both the hydroxyl and the amino group undergo many analogous chemical reactions, but in the animal body their behaviour is quite distinct, for the amino group does not conjugate with either glucuronic acid or sulphuric acid.

The types of amino group which are acetylated *in vivo* are the aromatic amino group and the amino group of the α-amino acids. A typical example of the former is sulphanilamide, which is largely converted *in vivo* to *p*-acetaminobenzenesulphonamide :—

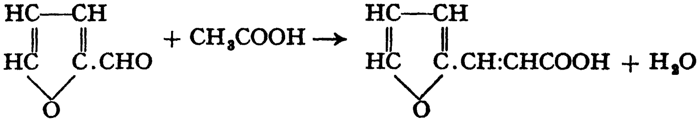


Examples of the latter type are γ-phenyl-α-aminobutyric acid and *p*-bromophenyl-*l*-cysteine ; these are partly converted to the corresponding N-acetyl derivatives :—

$$C_6H_5CH_2CH_2CH(NH_2)COOH \rightarrow C_6H_5CH_2CH_2CH(NHCOCH_3)COOH$$
$$p\text{-}BrC_6H_4SCH_2CH(NH_2)COOH \rightarrow p\text{-}BrC_6H_4SCH_2CH(NHCOCH_3)COOH$$

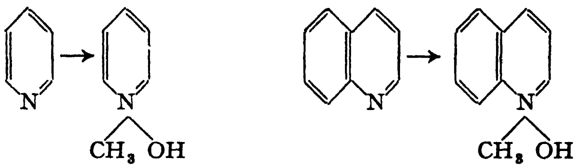
Acetylation of the aromatic amino group takes place in all species of animals studied (including the hen), with the exception of the dog, which can however acetylate the amino acid $NH_2$ group. These facts indicate that the acetylation mechanism of aromatic amines is probably different from that involved in the acetylation of amino-acid derivatives (Stekol, **1941**).

It seems probable also that acetic acid is involved in the metabolism of furfural, for this aldehyde when fed to rabbits and dogs is partly excreted as furfuracrylic acid. It is considered that the formation of this unsaturated derivative is the result of a reaction analogous to the Perkin synthesis, thus :—



There is, however, no definite experimental evidence to support this mechanism. Furfural is unique in this respect for no other aldehyde has been found to undergo this change in the animal body.

8. **Methylation.** Methylation is probably a much more widespread reaction in the animal body than has hitherto been thought (see du Vigneaud, 1941). From the point of view of detoxication mechanisms, however, it has been found that methylation is confined to the heterocyclic nitrogen atom in compounds of the pyridine and quinoline type. Pyridine and quinoline, for example, are partly converted by most animals, except the rabbit, to N-methylpyridinium hydroxide and N-methylquinolinium hydroxide respectively :—



Other heterocyclic rings containing nitrogen, e.g. imidazole and piperidine, are not methylated (but see p. 153).

9. **The Thiocyanate Detoxication.** This process is said to take place when inorganic cyanides are administered to animals or when the —CN group from organic cyanides is hydrolysed *in vivo* to HCN. The CN′ radicle is largely converted to the non-toxic thiocyanate radicle CNS′. This process is a true detoxication, for whereas 3 mg./kg. of HCN will kill a rabbit, as much as 500 mg./kg. of NaCNS is required. It is probable that the greater part of administered HCN is transformed into thiocyanate which is excreted in the urine (Lang, 1894). Sodium thiocyanate is not metabolised in the animal and according to Pollak (1902), when fed to men or dogs or injected into rabbits it is excreted unchanged almost quantitatively.

## HISTORICAL

The first biochemical reaction to be discovered which is today classified as a " detoxication mechanism " was the synthesis in the mammalian organism of hippuric acid from ingested benzoic acid. This discovery was made over a hundred years ago for the first unequivocal proof of the occurrence of this synthesis was provided in 1842 by Keller.

According to Kingzett (1878) hippuric acid was discovered in 1784 by Rouelle, who found it in the urine of cows. However, hippuric and benzoic acids were not properly differentiated by these early chemists, and Berzelius (1840) makes the following statement about Rouelle's observation. " Rouelle fand, dass Salzsäure aus einem durch Abdampfen concentrirten Kuhharn ein säures Salz fällte, welches er mit Benzoëblumen verglich." This confusion is apparent also in statements made in an early textbook of chemistry by Thompson (1802) regarding the occurrence of benzoic acid in animal urine. This author states that Scheele evaporated urine to dryness, separated the saline part and applied heat to the residuum whereby benzoic acid sublimed, but that Proust (1801 ; cf. Fourcroy and Vauquelin, 1799) was of the opinion that the acid obtained by Scheele's process was not benzoic acid but another acid of similar properties which was decomposed by nitric acid which only " whitens benzoic acid ". There is little doubt that the recognition of hippuric acid as a separate entity from benzoic acid was made by Liebig about 1829 (according to Keller, see below). Hofman in his Faraday Lecture [1] on Liebig delivered before the Chemical Society of London in 1875 makes the following statement regarding the discovery of hippuric acid by Liebig.

> In 1830, Liebig repeats the experiments of Rouelle, as well as the later ones of Fourcroy and Vauquelin, which had indicated the presence of benzoic acid in the fluid secretions of horses. These experiments lead him to the discovery of an acid containing nitrogen, to which he gives the name hippuric acid. He remarks that it may be considered as a compound of benzoic acid with an organic body as yet unknown. The nature of this body was reserved for Dessaignes to establish, by showing that hippuric acid, when treated with acids, splits up into benzoic acid and glycocoll.

The first clear-cut proof of the synthesis of hippuric acid from ingested benzoic acid was published by Keller (1842), who worked in Wöhler's laboratory at Göttingen. It is often claimed that Wöhler discovered this synthesis in 1824 (see Berzelius, 1831, cited by Bunge, 1890 ; cf. also Lewis, 1941), but we see from Keller's statement quoted below that Wöhler confused benzoic acid with hippuric acid, although the possibility of the conversion of benzoic acid to hippuric acid *in vivo* had occurred to him. A full English translation of his paper which appeared in German in *Annalen der Chemie und Pharmacie* is published in the appendix to Liebig's book *Animal Chemistry or Organic Chemistry in its applications to Physiology and Pathology* (1842). The first two paragraphs of the translation of Keller's paper which is entitled " On the conversion of benzoic acid into hippuric acid " read as follows :

> So early as in the edition of Berzelius's *Lehrbuch der Chemie*, published in 1831, Professor Wöhler had expressed the opinion, that benzoic acid, during digestion, was probably converted into hippuric acid. This opinion was founded on an

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[1] " The Life-work of Liebig in Experimental and Philosophic Chemistry ; with allusions to his Influence on the Development of the Collateral Sciences, and of the Useful Arts " (Hofman, 1875).

> experiment which he had made on the passage of benzoic acid into the urine. He found in the urine of a dog which had eaten half a drachm of benzoic acid with his food, an acid crystallizing in needle-shaped prisms, which had the general properties of benzoic acid, and which he then took for benzoic acid. These crystals were obviously hippuric acid, as plainly appears from the statements, that they had the aspect of nitre, and, when sublimed, left a residue of carbon. But at that time hippuric acid was not yet discovered; and it is well known, that till 1829, when these acids were first distinguished from each other by Liebig, it was uniformly confounded with benzoic acid.
>
> The recently published statement of A. Ure,[1] that he actually found hippuric acid in the urine of a patient who had taken benzoic acid, recalled this relation, so remarkable in a physiological point of view, and induced me to undertake the following experiments, which, at the suggestion of Professor Wöhler, I made on myself. The supposed conversion of benzoic acid into hippuric acid has, by these experiments, been unequivocally established.

Camel (Schwartz, 1845) and cow urine (Kraut, 1858) were also found to be rich sources of hippuric acid. It was not, however, until 1867 that it was proved that other aromatic acids might undergo the hippuric acid synthesis *in vivo*, when Schultzen and Gräbe (1867) showed that the substituted benzoic acids such as chlorobenzoic and anisic acids formed the corresponding hippuric acids *in vivo*.

The next mechanism to be discovered was the oxidation of benzene to phenol (1867), which led eventually to the discovery of the ethereal sulphates (1876). Städeler (1851) had found that cow urine when distilled with sulphuric acid yielded phenols. He considered that the phenols were present in cow urine in combination as an acid which he called "Taurylsäure" (probably *p*-cresylsulphuric acid). In 1867 Schultzen and Naunyn discovered that if benzene were administered to either men or dogs phenol appeared in the urine, a fact which was confirmed by Munk in 1876. Furthermore, Schultzen and Naunyn (1867) found that toluene was oxidised *in vivo* to benzoic acid and xylene to toluic acid, thus establishing the fact that aromatic substances could be oxidised in the animal body.

The twenty years between 1875 and 1895 saw the discovery of the majority of the so-called detoxication mechanisms with the exception of the glutamine synthesis in man (1914). These discoveries were closely connected with the development of organic chemistry. Frequently the discovery of a new type of compound was followed by a study of its physiological properties and fate in the body. At that time much interest was focused on the indigo-forming substances in human and, particularly, in herbivorous urines. It had been observed that both normal and pathological urines sometimes gave on standing a bluish sediment which was identified as indigo. During an investigation of the indigo-forming substances of urine in Hoppe-Seyler's laboratory, Baumann found that they were decomposed by glacial acetic acid or hydrochloric acid to give a certain amount of sulphuric acid. He concluded that these substances were "paired" sulphuric acids with a carbon containing complex of unknown nature (Baumann, 1876*a*). He also found that in relation to the inorganic sulphate these paired sulphuric acids were much more abundant in horse than in human urine. Eventually Baumann (1876*b*) was able to demonstrate that the paired sulphuric acids were compounds of phenol, catechol and the indigo-forming substances with sulphuric acid. Potassium phenylsulphate was isolated and shown to be present in large quantities in the urine of a patient treated with carbolic acid. Baumann also found a marked increase in the conjugated sulphate in the urine of a dog which had received a 2 g. dose of catechol.

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[1] This work of Ure is referred to in the *London Medical Gazette* (1841) as follows :—

"*On Gouty Concretions; with a New Method of Treatment*

"The author's intention, in the few remarks contained in this paper, is to introduce to the profession a remedy which he believes to be likely to prevent the formation of tophous concretions in gouty subjects. The remedy in question is benzoic acid, administered in doses of one scruple an hour after a meal. In the course of a couple of hours (as the author has found by frequent experiments made upon himself and others), the urine voided, amounting to five or six ounces, will be found, on adding a small quantity of muriatic acid, to yield a copious precipitate of beautiful rose-pink acicular crystals, which weigh, after being allowed to settle for a day, about fifteen grains. The body thus produced by the agency of vital chemistry is hippuric acid, and is found to have taken the place of uric acid in the urine, more of the latter being discoverable. By thus substituting hippurate of soda, a salt of easy solubility, for the very sparingly soluble urate of that alkali, the author conceives that the formation of the tophous concretions may be altogether prevented."

It is interesting to note that earlier chemists had observed that two forms of sulphates occurred in the urine. In an early textbook called *The Elements of Experimental Chemistry*, Henry (1829) makes the following statement regarding the sulphates in urine :—

> The existence of salts, containing sulphuric acid, in urine is proved by adding muriate of baryta, to urine acidulated with muriatic acid. This excess of acid prevents the precipitation of the phosphates, which would otherwise be decomposed by the barytic salt. From the weight of the precipitate, Berzelius computes that the proportion of sulphuric acid in urine exceeds that of phosphoric acid. If nitrate of baryta, with an excess of nitric acid, be employed, and if the urine, after depositing the sulphate of baryta, be evaporated, a further portion of sulphate of baryta is deposited in small hard crystals. Now the sulphuric acid, which occasions this *second* [1] production of the barytic sulphate, must have been *formed* during evaporation ; and can only be accounted for by supposing that a portion of sulphur, existing in the urine, has been acidified by the excess of nitric acid.

Baumann (1876*a*) then discovered that the alkali salts of phenylsulphuric acid were practically non-toxic, and it is about this time that the conception of detoxication began to appear. Workers at that time recognised these processes simply as biological syntheses, conjugations and oxidations rather than as mechanisms for the reduction of the toxicity of a compound, and the idea of detoxication was not fully developed until much later. We find, for example, that Bunge, in his textbook *Physiological and Pathological Chemistry* (English trans., London, 1890), whilst discussing these syntheses and oxidations at length does not once refer to them as true detoxication processes, yet Neumeister in his

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[1] Author's italics.

*Lehrbuch der physiologische Chemie* (1895) refers to glucuronic acid combining with foreign compounds or their oxidation products, which thereby underwent " detoxication " (*Entgiftung*), a means of removing injurious compounds from the body.

A number of investigators had noticed that, on feeding certain compounds (chloral hydrate, nitrobenzene, *o*-nitrotoluene, bromobenzene, chloroform, morphine) to animals, the resulting urines were often strongly laevorotatory and reduced, particularly after hydrolysis, alkaline copper and bismuth solutions on warming. The substances responsible for these properties were also non-fermentable by yeast, a fact which seemed to rule out glucose. Mering and Musculus (1875) found that following the administration of chloral hydrate the urine of humans contained a compound which was strongly acidic, copper reducing and laevorotatory. This compound was isolated in crystalline form and named urochloralic acid. At about the same time Jaffe (1874), investigating the behaviour of isomeric compounds in the animal body, obtained the first clear indication of the existence of glucuronic acid. At this point it should be mentioned that in 1855 Schmid found that euxanthic acid (which we now know to be euxanthone glucuronide) yielded on hydrolysis by acid a copper-reducing substance. This was confirmed by Baeyer (1870), who stated that, on the basis of the equation for the formation of euxanthone from euxanthic acid, the reducing substance should have the formula $C_6H_{10}O_7$, which as we know now is correct for glucuronic acid. Baeyer was of the opinion that the reducing substance was " a kind of saccharic acid ". Baumann in 1877 observed that after the administration of indole to animals the urine contained besides indoxylsulphuric acid, an indole-containing compound which was not an ethereal sulphate but which contained indole combined with an organic radicle, now known to be glucuronic acid.

At about the same period Jaffe (1874, 1878) investigated the fate of *p*-nitrotoluene and *o*-nitrotoluene in the dog. He found that the former was mainly oxidised to *p*-nitrobenzoic acid and excreted combined partly with glycine, but with the *ortho* compound only about 10% was oxidised to the acid and unlike the *m*- and *p*-isomerides, this nitrobenzoic acid did not form nitrohippuric acid. The main product of *o*-nitrotoluene metabolism in the dog was a substance which Jaffe called " Uronitrotoluolsäure ", $C_{13}H_{15}NO_9$, the yield of which corresponded to 25% of the *o*-nitrotoluene fed. It was isolated from the urine in combination with urea and was found to be strongly laevorotatory and acidic, and on hydrolysis was a reducing agent. Its properties suggested that it might be glucosidic, i.e. a compound of *o*-nitrobenzoic acid and glucose, which would break down thus :—

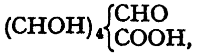
$$C_{13}H_{15}NO_9 + H_2O = C_7H_5NO_4 + C_6H_{12}O_6$$

On hydrolysis with dilute sulphuric acid, however, it gave the then unknown *o*-nitrobenzyl alcohol and not *o*-nitrobenzoic acid. Removal of the alcohol left a small residue which was laevorotatory, reducing, acidic and non-fermentable by yeast. Jaffe therefore represented the hydrolysis of " Uronitrotoluolsäure " thus :—

$$C_{13}H_{15}NO_9 + H_2O = C_7H_7NO_3 + C_6H_{10}O_7$$

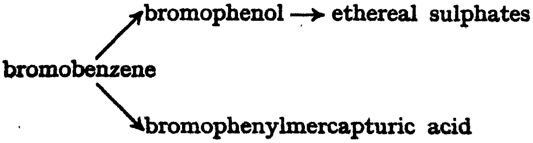
The syrupy residue after removal of the alcohol contained a " hypothetical acid ", $C_6H_{10}O_7$, which Jaffe considered to be carbohydrate in nature, an aldehyde-acid which was an oxidation product of a sugar in which the —$CH_2OH$ group had been converted to —COOH. He also pointed out that this acid was probably a component of Mering and Musculus's " Urochloralsäure ", a fact later confirmed by Mering (1882).

In the year following the publication of Jaffe's paper on the metabolism of *o*-nitrotoluene, Schmiedeberg and Mayer (1879) isolated glucuronic acid (or, to be precise, its lactone, glucurone) and suggested that it was identical with Jaffe's hypothetical acid. It was found as a component of the camphoglycuronic acids excreted by dogs which had ingested camphor. From its properties they deduced its structure as being



$$(CHOH)_4\begin{cases}CHO\\COOH,\end{cases}$$

and named it " Glycuronsäure ". However, unlike Jaffe's " hypothetische Säure ", it had a dextrorotation in aqueous solution equal to about one-half that of glucose. The discrepancy regarding the direction of rotation of Jaffe's " hypothetische Säure " and Schmiedeberg and Mayer's " Glycuronsäure " was explained by the latter workers as being due to the fact that Jaffe's crude acid was contaminated with the highly laevorotatory " Uronitrotoluolsäure ". Schmiedeberg and Mayer also expressed views regarding the origin of glucuronic acid in the organism, and stated that it probably represented an intermediate stage in the combustion of sugar (presumably glucose) in the body, but by pairing with camphor the sugar escaped further decomposition. The same year as Schmiedeberg and Mayer announced their discovery of glucuronic acid in the urine of camphor-fed dogs, the mercapturic acid synthesis was discovered by two independent groups of workers. Baumann, continuing his work on the compounds which resulted in the increased excretion of ethereal sulphates, found (Baumann and Preusse, 1879) that bromobenzene not only increased the ethereal sulphate output in animals (i.e. from bromophenol) but also caused the excretion of another sulphur-containing compound, namely, *p*-bromophenylmercapturic acid. This discovery was also made independently by Jaffe (1879), who showed that chloro- and iodobenzene also formed mercapturic acids *in vivo*. Baumann (1883) expressed the view that the formation of bromophenol and its ethereal sulphate was independent of the mercapturic acid synthesis thus :—



This view has been supported by more recent investigations. Baumann's opinion that mercapturic acids are also excreted combined with glucuronic acid has, however, not been demonstrated experimentally and is probably erroneous.

The close chemical analogies between certain heterocyclic compounds and benzene derivatives led Jaffe and his pupils to investigate their fate in the animal in order to find out whether this analogous behaviour persisted in the biochemical sphere. Furfural was fed to dogs and rabbits and whereas it was found that this compound behaved partly in a manner similar to benzaldehyde in that it was oxidised to pyromucic acid and excreted as furoylglycine, it also formed furfuracryluric acid (Jaffe and Cohn, 1887). Jaffe expressed the opinion that furfural had undergone *in vivo* a change analogous to the Perkin synthesis of cinnamic acid from benzaldehyde and sodium acetate. This reaction also showed that acetic acid could take part in biological syntheses. The acetylation of aniline was discovered at about the same time (see below). In order to find out whether the acrylic acid synthesis occurred in animals other than the dog and rabbit, Jaffe and Cohn (1888) fed furfural to hens and found that they excreted neither furoylglycine nor furfuracrylic acid, although they oxidised furfural to pyromucic acid. The only other metabolite found was furfurornithuric acid. The discovery of the ornithuric acid synthesis in hens had been made by Jaffe (1877) some ten years earlier, when he found that if benzoic acid were fed to hens it was excreted as ornithuric acid (dibenzoylornithine) and not as hippuric acid.

The year 1887 also saw the discovery of the biological methylation of the heterocyclic nitrogen atom of the pyridine type of compound, for His (1887) showed that pyridine administered to dogs was excreted as methylpyridinium hydroxide.

In 1893 Cohn published an account of his efforts to find out if the acrylic acid synthesis took place with aldehydes other than furfural and discovered the process of acetylation of the aromatic amino compounds. Schmiedeberg (1878) had earlier discovered that aniline was oxidised *in vivo* to *p*-aminophenol and excreted as an ethereal sulphate, and Müller (1887) had found that acetanilide was excreted by man as *p*-acetylaminophenylsulphate. Müller discovered the first acetylated compound, but probably did not realise that acetanilide was first deacetylated to form aniline, which was then oxidised and acetylated to give *p*-acetaminophenol. Cohn fed the *o*-, *m*- and *p*-nitrobenzaldehydes to dogs and rabbits and found that the *m*- and *p*-compounds were excreted as acetylaminobenzoic acids. He considered the first changes to be the formation of aminobenzoic acids by oxidation and reduction, which were then followed by acetylation, a reaction analogous to the formation of furfuracrylic acid which Cohn formulated as follows:—

$$C_4H_3O.CHO + CH_3COOH = C_4H_3O.CH{:}CHCOOH + H_2O$$

$$C_6H_4(COOH)NH_2 + CH_3COOH = C_6H_4(COOH)NHCOCH_3 + H_2O$$

Chronologically the next detoxication mechanism to be brought to light was the thiocyanate mechanism. Lang (1894) discovered that about one-fifth to one-sixth of small doses of hydrocyanic acid administered to a dog was excreted in the urine as non-toxic thiocyanates. Hydrocyanic acid is nearly 200 times more toxic than thiocyanate and the conversion of the former into the latter appears to be a true detoxication process.

The last of the detoxication mechanisms to be discovered was the glutamine synthesis. This discovery was made in the present century when Thierfelder and Sherwin (1914, 1915) found that in man phenylacetic acid was excreted as phenylacetylglutamine. The only other animal which can carry out this synthesis is the related chimpanzee (Power, 1936).

Whether other so-called detoxication mechanisms of the synthetic type will be discovered in the future has yet to be seen, but it is interesting to note that during the last half-century only one new synthesis, the glutamine conjugation, has been found. It is possible that new mechanisms may be found, particularly when the study of comparative biochemistry is expanded, for our knowledge of the fate of organic compounds in the organism is confined to but a few species.

# *Chapter Two*

# The Metabolism of some Aliphatic Compounds and Cyclohexane Derivatives

## ALIPHATIC ALCOHOLS

### Primary Alcohols

PRIMARY aliphatic alcohols are considerably degraded in the body and only small amounts of their metabolites find their way into the urine. The corresponding aldehydes and fatty acids are intermediates in this degradation and the oxidation can probably be represented as follows :—

$$R.CH_2OH \rightarrow R.CHO \rightarrow R.COOH \rightarrow CO_2 \text{ and } H_2O$$

From a qualitative study of the fate of the lower primary alcohols in the rabbit and the dog, Neubauer (1901) showed by means of tests (orcinol test, reduction test and polarimetry) of doubtful reliability that ethyl, *n*-propyl, *n*-butyl, primary *iso*butyl ($(CH_3)_2CHCH_2OH$), and *n*-octyl alcohols and *iso*butylcarbinol ($(CH_3)_2CHCH_2CH_2OH$) cause an increased excretion of glucuronic acid in both animals. Methyl alcohol apparently does not increase the glucuronic acid output (Neubauer, 1901 ; Pohl, 1893). The tendency of primary alcohols to form glucuronides is greater in the rabbit than in the dog.

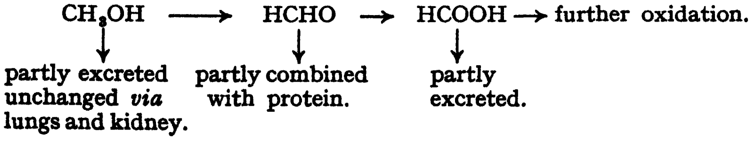
**Methyl Alcohol.** Methyl alcohol is oxidised in the animal body at a relatively slow rate compared with ethyl alcohol. According to Widmark (1933) it is metabolised in rabbits about five times as slowly as ethyl alcohol, and in dogs only 39% (at a dose level of 2 c.c./kg.) is oxidised in 48 hours (Völtz and Dietrich, 1912). This slow rate of oxidation indicates the possibility of rapid accumulation and of delayed toxic effects. The nature of the metabolites of methyl alcohol is of great importance in explaining the effects of the alcohol upon the organism. It is claimed that the toxic action of the alcohol is largely due to its metabolites, whereas its narcotic action which is characteristic of primary alcohols, is specifically due to the unchanged alcohol. To deal with the narcotic action first, it has been found that with methyl alcohol the narcotic effect is less than that of its higher homologues, possibly due to its lower solubility in lipoids and to greater miscibility with water. The partition coefficient, oil/water, for methyl alcohol is 2·5/100 (Loewy and von der Heide, 1914).

The immediate metabolites of methyl alcohol are formaldehyde and formic acid, both of which are highly toxic compared with the parent substance. This greater toxicity of the oxidation products of methyl alcohol is well illustrated by their effects on the isolated frog's heart, for Simon (1933) has shown that the heart is reversibly arrested by 200 g./litre (6·25 moles/l.) of methyl alcohol but irreversibly arrested by 1 g./litre (0·0333 moles/l.) of formaldehyde and by 0·2 g./litre

(0·00434 mole/l.) of formic acid. The detection of formaldehyde as a metabolite of methyl alcohol is difficult since it reacts very promptly with proteins, but both Pohl (1893) and Keeser (1931) claim to have detected it in the tissues of methyl alcohol poisoned animals, the former by the unspecific fuchsin reaction and the latter by formation of hexamethylenetetramine and the colour reaction of formaldehyde with resorcinol and caustic soda. Numerous workers have shown that there is an increased excretion of formate after the ingestion or inhalation of methyl alcohol (e.g. Pohl, 1893; Rost and Braun, 1926; Hunt, 1902; Bachem, 1927). Some methyl alcohol is eliminated unchanged through the lungs for Völtz and Dietrich (1922) found that dogs exhale 15·3% of a dose of 2 c.c./kg. in 24 hours, whereas only 1·5% is excreted in the urine. When repeated doses of methyl alcohol are given to dogs, they acquire some tolerance to the alcohol and the excretion of formate decreases, probably due to greater excretion of the unoxidised alcohol (Leo, 1927) and not to greater ability to oxidise formate, for dogs accustomed to methyl alcohol excrete about the same amount of ingested sodium formate as unaccustomed dogs (Leo, 1927).

One of the more serious symptoms of methyl alcohol poisoning is the effect on vision. Repeated doses can cause total and permanent blindness both in man and animals. This effect is thought to be caused by its metabolites, formaldehyde and formic acid. Keeser (1931) has detected formaldehyde in the aqueous and vitreous humour of methyl alcohol poisoned rabbits, and has shown that the fresh vitreous humour of the calf can oxidise methyl alcohol to formaldehyde *in vitro*. The retinas of poisoned animals appear to be unable to utilise oxygen (Goldschmidt, 1922). The vitreous humour, which is less effectively buffered than blood, increases in acidity, and it is possible that more formic acid is formed in this organ than in others because here formaldehyde combines less readily with protein (see von Oettingen, 1943). According to Roe (1943), however, the formic acid produced inhibits iron-containing respiratory systems, resulting in the accumulation of lactic acid. Formic acid may also interfere with the oxidative processes of the retinal cells.

The metabolism of methyl alcohol in the animal body can probably be represented as follows:



$$\begin{array}{ccccc} CH_3OH & \longrightarrow & HCHO & \longrightarrow & HCOOH \longrightarrow \text{further oxidation.} \\ \downarrow & & \downarrow & & \downarrow \\ \text{partly excreted} & & \text{partly combined} & & \text{partly} \\ \text{unchanged } \textit{via} & & \text{with protein.} & & \text{excreted.} \\ \text{lungs and kidney.} & & & & \end{array}$$

Methyl alcohol is said to be a metabolite of **methyl chloride** (Gross, 1934), a substance used as a refrigerant. Persons suffering from methyl chloride poisoning excrete formates and display the characteristic toxic effects of methyl alcohol. The fate of methyl chloride is probably as follows:—

$$CH_3Cl \rightarrow HCl + CH_3OH \rightarrow HCHO \rightarrow HCOOH$$

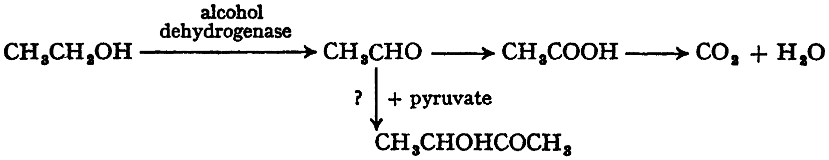
**Ethyl Alcohol.** The work of Atwater and Benedict (1899) showed that alcohol was quickly destroyed in the body, not more than 2% being eliminated undecomposed. These workers found no evidence that any metabolites other than carbon dioxide and water were eliminated. However, later work with isolated tissues has shown that both acetaldehyde and acetic acid are formed during the oxidation of alcohol (Battelli and Stern, 1910). Oxidation of alcohol to the acetic acid stage takes place in the liver (Leloir and Muñoz, 1938), whereas further stages involving complete oxidation occur in other tissues, particularly the muscles (Lundsgaard, 1938).

That acetaldehyde is the primary product of the metabolism of ethyl alcohol in the animal body has now been definitely established, for Stotz (1943) has shown that, after the administration of alcohol to man, the acetaldehyde concentration of the blood rises many-fold. Furthermore, alcohol dehydrogenase, an enzyme which converts alcohol to acetaldehyde, is found in the liver. In dogs the rate of disappearance of alcohol from the blood is increased by the oral administration of pyruvate, and that of pyruvate by the administration of alcohol. Westerfeld, Stotz and Berg (1942) suggested that this effect may be due to the condensation of acetaldehyde, the primary oxidation product of alcohol, with pyruvate to form acetoin, $CH_3CHOHCOCH_3$. However, since administered acetoin is only slowly metabolised and does not accumulate during alcohol metabolism (cf. Greenberg, 1942 ; 1943), Westerfeld, Stotz and Berg (1943) put forward an alternative suggestion in which they state that the increased rate of alcohol metabolism caused by oral administration of pyruvate is due to a coupled oxidation-reduction reaction involving both the oxidation of alcohol to acetaldehyde and simultaneous reduction of pyruvate to lactate. In a later paper, however, Stotz, Westerfeld and Berg (1944) still envisage the possibility of alcohol forming acetoin *in vivo*, for they were able to show that acetaldehyde, a metabolite of alcohol, on injection into rats causes a rise in the blood acetoin (see p. 25). The conversion of acetaldehyde and pyruvate into acetoin requires the presence of thiamine (cf. Berg, Stotz and Westerfeld, 1944). Results, however, have been obtained by Gregory, Ewing and Duff-White (1943) which apparently contradict those of Westerfeld *et al.*, for the former workers found no evidence that pyruvate increases the rate of metabolism of ethyl alcohol.

The elimination of acetic acid after the feeding of alcohol to rabbits has been demonstrated by Bernhard (1940), using deuterated alcohol. Deuteroethyl alcohol is excreted in the urine to the extent of 24% as deuteroacetic acid.

Neubauer (1901) has claimed that ethyl alcohol causes a small increase in the output of conjugated glucuronic acid in the rabbit. This glucuronide might be ethylglucuronide or, if the views of Westerfeld *et al.* are correct, the glucuronide of acetoin, for this substance when fed to rabbits is excreted partly as a glucuronide (Neuberg and Gottschalk, 1925 ; see p. 22).

The metabolism of ethyl alcohol in the animal body may be represented as follows :—



$$\begin{tikzcd} CH_3CH_2OH \arrow[r, "\text{alcohol dehydrogenase}"] & CH_3CHO \arrow[r] \arrow[d, "?"', "+\text{ pyruvate}"] & CH_3COOH \arrow[r] & CO_2 + H_2O \\ & CH_3CHOHCOCH_3 & & \end{tikzcd}$$

In the case of substituted derivatives of ethyl alcohol it is to be expected that the rate of oxidation *in vivo* will be slow, and under such circumstances excretion of glucuronides, if they are formed, should be appreciable. This is actually the case when trichloro- and tribromoethyl alcohols are fed. Both these alcohols are used in surgery as basal anaesthetics.

**Tribromoethyl alcohol** is used under the name " avertin ". It is excreted by the animal organism in the urine as tribromoethylglucuronide, a compound which was isolated by Endoh (1924) from the urine of rabbits receiving the alcohol orally.

$$CBr_3CH_2OH \rightarrow CBr_3CH_2OC_6H_9O_6$$

The glucuronide is a strongly acidic and hygroscopic substance (m.p. 145·5°) which reduces Fehling's solution in virtue of the presence of the unstable configuration of three halogen atoms attached to the same carbon atom. The amount of glucuronic acid in the urine of patients treated with avertin is said to be a valuable indication of its rate of excretion and detoxication (Walter, 1931).

**Trichloroethyl alcohol** is excreted partly unchanged and partly as trichloroethylglucuronide (urochloralic acid). As an anaesthetic it appears to possess a wider margin of safety than " avertin " since smaller fractions of the fatal dose are needed to produce sleep and anaesthesia (Lehmann and Knoefel, 1939).

***n*-Propyl alcohol** is more readily oxidised than ethyl and methyl alcohols and is apparently completely oxidised, no intermediates being detectable (Neymark, 1938 ; Weese, 1928).

***n*-Butyl alcohol** is completely oxidised in mice (Weese, 1928) and its rate of oxidation is more rapid than that of ethyl alcohol (Berggren, 1938). ***Iso*butyl alcohol** is also completely oxidised in mice (Weese, 1928). ***n*-Amyl alcohol** is mainly oxidised *in vivo*, although some 5–10% may be excreted unchanged by the lungs and kidney. Intermediary products of the oxidation of propyl, butyl and amyl alcohols are not known.

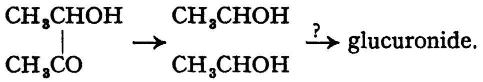
**Cetyl alcohol** (*n*-hexadecyl alcohol) is oxidised in the rat to the corresponding fatty acid, palmitic acid (Stetten and Schoenheimer, 1940).

### Secondary Alcohols

It could be expected that, if primary alcohols are oxidised in the body to acids, then secondary alcohols would probably form ketones. In the case of ***iso*propyl alcohol**, Neymark (1938) demonstrated its quantitative transformation to acetone during its metabolism in the dog.

Secondary alcohols probably conjugate with glucuronic acid to some extent; thus Neubauer (1901) showed that *iso*propyl, methyl-*n*-propyl and methyl-*n*-hexyl (secondary capryl) alcohols increased the excretion of glucuronic acid in dogs and rabbits.

**Acetoin** (acetylmethylcarbinol). This substance is sometimes formed together with 2:3-butylene glycol during the fermentation of sugars by *B. coli* and other members of the *Bacteriaceae*. Both these substances can be oxidised to diacetyl, a substance which gives butter its characteristic flavour. When fed to dogs acetoin is excreted to the extent of 5–25% as 2:3-butylene glycol. Small amounts of unchanged acetoin are also excreted. No diacetyl is formed, and when fed to fasted rats, acetoin does not increase the amount of glycogen in the liver (Westerfeld and Berg, 1943). Neuberg and Gottschalk (1925) claim that it forms a glucuronide in rabbits, and this compound may be formed from either acetoin or its metabolite, 2:3-butylene glycol (see p. 24).



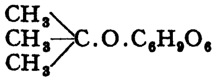
$$\begin{matrix}CH_3CHOH\\ |\\ CH_3CO\end{matrix} \rightarrow \begin{matrix}CH_3CHOH\\ \\ CH_3CHOH\end{matrix} \xrightarrow{?} \text{glucuronide.}$$

## Tertiary Alcohols

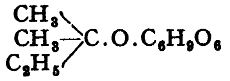
Tertiary alcohols are only oxidised with difficulty in the body, a fact which may possibly be correlated with the extreme branching of the hydrocarbon chain. Metabolic products are therefore excreted in the urine in appreciable amounts. In the cases of tertiary butyl and amyl alcohols, their conjugated glucuronic acids can be isolated from urine.

**Tertiary Butyl Alcohol** or trimethylcarbinol ($(CH_3)_3COH$), as could be expected, does not increase the output of ethereal sulphates in rabbits or in dogs. When fed to rabbits it is excreted as tertiary butylglucuronide ($[\alpha]_D - 28{\cdot}5°$ for the potassium salt in water).

Large doses (6 c.c.) have a strong narcotic action, causing rabbits to become drowsy for several hours. Dogs react differently from rabbits, since 10 c.c. of the alcohol given to a medium-sized dog cause no drowsiness and the character of the urine is normal (Thierfelder and Mering, 1885). The dog does not excrete a conjugated glucuronide as does the rabbit. Tertiary butylglucuronide, unlike most conjugated glucuronic acids, is not precipitated from solution by basic lead acetate. Tertiary amylglucuronide is similar in this respect.



tertiary butylglucuronide



tertiary amylglucuronide

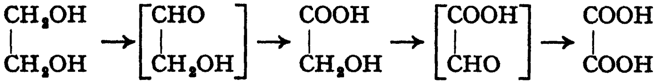
**Tertiary amyl alcohol** (amylene hydrate) when fed to rabbits, also results in the excretion of a glucuronide. In the dog, however, no glucuronide is excreted even after feeding 20 c.c. of the alcohol. Thierfelder and Mering found that a man who had taken 12 c.c. of the alcohol in two days or three doses of 3 c.c. in one day, did not excrete a glucuronide.

The crystalline potassium salt of tertiary amylglucuronide is similar in properties to the tertiary butyl compound; it is laevorotatory ($[\alpha]_D - 32{\cdot}1°$ in water) and non-reducing.

## THE GLYCOLS

The glycols are used in a wide variety of industries as solvents, particularly for cosmetics and pharmaceutical products. The fate of a number of these compounds in the animal body has been investigated, and it appears that they are oxidised with greater difficulty than the monohydric primary alcohols. In general, they are oxidised to the corresponding dicarboxylic acids and there is also in some cases a tendency to form conjugated glucuronic acids.

**Ethylene Glycol.** This compound is used a great deal as a solvent for dyes and drugs. It is utilised in the cosmetics and perfume industry and is valuable as an antifreeze in automobile radiators. Its monophenyl ether, phenoxetol, has antibacterial properties; *Pseudomonas pyocyanea*, a common invader of wounds which is resistant to penicillin, is particularly sensitive to phenoxetol *in vitro* (Berry, 1944). In the animal body ethylene glycol is oxidised, *via* glycollic acid, to oxalic acid; the other possible intermediates in the oxidation, glycollic aldehyde and glyoxalic acid, have not been detected (Pohl, 1896; Dakin, 1907; Bachem, 1917; Mayer, 1903).

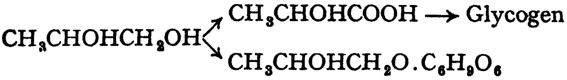


$$\begin{matrix}CH_2OH\\|\\CH_2OH\end{matrix}\rightarrow\begin{bmatrix}CHO\\|\\CH_2OH\end{bmatrix}\rightarrow\begin{matrix}COOH\\|\\CH_2OH\end{matrix}\rightarrow\begin{bmatrix}COOH\\|\\CHO\end{bmatrix}\rightarrow\begin{matrix}COOH\\|\\COOH\end{matrix}$$

The quantity of ethylene glycol excreted as oxalic acid by dogs and rabbits is small and figures varying from 0·4% to 2·3% have been recorded by different authors. Intravenous injection of sublethal single doses *ca.* 2·5 c.c./kg. of ethylene glycol or its diacetate into rats or rabbits results in the deposition of crystalline calcium oxalate in the renal tubules, and there is an accompanying haematuria. Toxic doses, i.e. *ca.* 5 c.c./kg., do not, however, affect the kidneys (Mulinos, Pomerantz and Lojkin, 1943). Oral administration of 1–2% solutions of sodium oxalate to rats does not result in the deposition of calcium oxalate in the kidney and, according to Mulinos *et al.* (1943), this indicates that the acutely toxic effects of intravenous doses of ethylene glycol are due to causes other than the production of oxalate by oxidation. It does not form a glucuronide (Miura, 1911*a*).

**Propylene glycol,** $CH_3CHOHCH_2OH$, has similar uses to ethylene glycol. About one-half is excreted by dogs unchanged in the urine, the rest being rapidly oxidised probably to lactic acid (Lehman and Newman, 1937). Miura (1911*b*) has isolated from the urine of rabbits a propylene glycol glucuronide as its barium salt and suggested for it the formula, $CH_3CHOH\,CH_2O.C_6H_9O_6$. The more recent work of

Newman, Van Winkle, Kennedy and Morton (1940) has shown that when blood containing propylene glycol is perfused through cat liver there is a deposition of glycogen. The glycogen is probably formed from the lactic acid produced by oxidation of the glycol, for it is well known that lactic acid can form liver glycogen. The possible fate of propylene glycol may, therefore, be represented as follows:—



$$CH_3CHOHCH_2OH \begin{cases} \nearrow CH_3CHOHCOOH \rightarrow \text{Glycogen} \\ \searrow CH_3CHOHCH_2O.C_6H_9O_6 \end{cases}$$

Its isomeride, **trimethylene glycol**, $CH_2OHCH_2CH_2OH$, has little commercial use owing to its toxicity, which is twice that of propylene glycol. According to Van Winkle (1941) it is probably oxidised in the body to malonic acid and its toxicity may be attributed to the formation of this acid which forms insoluble calcium salts.

$$CH_2OHCH_2CH_2OH \rightarrow COOHCH_2COOH$$

Malonates are powerful inhibitors of several enzyme systems (e.g. succinic dehydrogenase) in the body, a fact which may also contribute to the toxicity of trimethylene glycol. In further contrast with propylene glycol, trimethylene glycol does not form liver glycogen *in vivo.*

**1:3-Butylene glycol,** $CH_3CHOHCH_2CH_2OH$, has a low toxicity, but the products of its metabolism are not known. Its isomeride, **2:3-butylene glycol,** $CH_3CHOHCHOHCH_3$, is largely metabolised in the dog. Some 12–14% is excreted unchanged and a small amount is conjugated; it forms neither liver glycogen nor diacetyl (Westerfeld and Berg, 1943). In the rabbit it forms a conjugated glucuronic acid (Neuberg and Gottschalk, 1925). **Pinacol,** $(CH_3)_2COHCOH(CH_3)_2$, is excreted as a glucuronide by the rabbit (Thierfelder and Mering, 1885).

**Panthenol** is of interest because of its relation to the B vitamin, pantothenic acid. When injected into rats or eaten by man it is partly oxidised to pantothenic acid which is excreted in the urine (Burlet, 1944).

$$HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2CH_2OH \rightarrow HOCH_2C(CH_3)_2CHOHCONHCH_2CH_2COOH$$

In man the urinary excretion of pantothenic acid after the ingestion of the alcohol is 50–100% greater than after the ingestion of calcium pantothenate. When pantothenic acid itself is ingested or injected parenterally 52–74% can be recovered unchanged in the urine (Pearson, 1941). Unlike pantothenic acid, panthenol has no effect on the growth of *Lactobacillus casei in vitro* (Burlet, 1944).

## ALIPHATIC ALDEHYDES

The lower unsubstituted aliphatic aldehydes appear to be completely oxidised in the animal body, and in the majority of cases only traces of metabolic products are excreted in the urine. Cohn (1893), for example, was unable to detect any metabolite in the urine of rabbits which had received acetaldehyde and paraldehyde, whilst Boyland

(1940) found no metabolite of heptaldehyde in the urine of rats and rabbits after its oral administration. The aliphatic aldehydes are probably oxidised *in vivo* to the corresponding fatty acid which in normal circumstances would undergo the ordinary processes of β-oxidation to carbon dioxide and water. There is no definite evidence to show that unsubstituted aldehydes undergo reduction to the corresponding alcohols, although this process does occur in the case of the halogen substituted aldehydes (see p. 27). If reduction took place, then the excretion of glucuronides could be expected, but Neubauer (1901) found that the administration of *iso*butaldehyde, *iso*valeraldehyde and heptaldehyde to dogs and rabbits did not result in the increased excretion of glucuronides. The fate of the aliphatic aldehyde can probably be represented as follows :—

$$\text{R.CHO} \rightarrow \text{R.COOH} \xrightarrow{\beta\text{-oxidation}} CO_2 + H_2O$$

**Formaldehyde.** Formaldehyde is rapidly oxidised to formic acid in the body, and according to Pohl (1893) there is, in dogs receiving the aldehyde, a small rise in the formate output of the urine. There is also little doubt that some of the administered formaldehyde combines with tissue proteins (see p. 19).

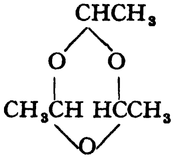
**Acetaldehyde.** It seems certain that acetaldehyde is oxidised *in vivo* to acetic acid (cf. ethyl alcohol). Stotz, Westerfeld and Berg (1944) have shown that it is quickly metabolised in homogenised rat brain, and that its rate of disappearance is greatly accelerated by addition of pyruvate and further increased by vitamin $B_1$ pyrophosphate (diphosphothiamine). The product of this reaction is acetoin which is formed almost quantitatively :—

$$CH_3CHO + CH_3COCOOH \rightarrow CH_3CHOHCOCH_3 + CO_2$$

The formation of acetoin from injected acetaldehyde can also be demonstrated *in vivo*. If rats are sacrificed 5 to 10 minutes after the last of several intraperitoneal injections of sublethal doses of acetaldehyde, acetoin is present in the blood (Stotz *et al.*, 1944), but if the period between killing the animals and the last injection of aldehyde is increased to 15–25 minutes acetoin is no longer detectable in the blood, and the blood acetaldehyde has reached a value less than 1 mg. per cent. This shows that acetaldehyde is metabolised extremely rapidly in the intact animal, producing in rats a state of intoxication similar to that produced by ethyl alcohol. Acetoin formation from acetaldehyde and hence from ethyl alcohol is not, however, of major significance in the metabolism of acetaldehyde.

There are five enzymes in the animal body which could be concerned with the metabolism of acetaldehyde, namely, xanthine oxidase, aldehyde oxidase, aldehyde mutase, aldolase and the animal carboxylase of Lubin and Westerfeld (1945). The first two enzymes which occur in the liver where the principal reactions involving acetaldehyde occur, oxidise it to acetic acid. Aldehyde mutase is unlikely to be concerned for ethyl alcohol, one of the products of acetaldehyde dismutation, has not been detected. Aldolase is probably not involved for its action occurs outside the liver, the main site of acetaldehyde metabolism. Animal carboxylase, a diphosphothiamine enzyme, condenses acetaldehyde and pyruvate to acetoin, a reaction which is only of minor significance. It has been reported (Knoop and Jost, 1924) that blood lactate increases after acetaldehyde. This could be the result of the action of aldehyde mutase with coenzyme I to produce a coupled reaction between acetaldehyde and pyruvate resulting in acetate and lactate (Lubin and Westerfeld, 1945).

**Paraldehyde.** Strictly speaking, paraldehyde is not an aldehyde but a heterocyclic compound formed by the polymerisation of three molecules of acetaldehyde.



paraldehyde

It is one of the oldest aliphatic hypnotics known to medicine. When given internally it imparts to the breath a strong odour which often persists for a whole day even after a single dose, and this fact led to the suggestion that it is mainly eliminated *via* the lungs. However, recent work has shown that a large proportion of administered paraldehyde is destroyed in the body and it has been suggested that it first undergoes depolymerisation to acetaldehyde, which is then metabolised as stated in the preceding section. This latter suggestion is supported by the fact that paraldehyde can provide the two-carbon residue necessary for the *in vivo* acetylation of sulphanilamide, for the amount of acetylsulphanilamide excreted by mice receiving sulphanilamide and paraldehyde simultaneously is greater than that excreted when sulphanilamide is administered alone (Hitchcock and Nelson, 1943). In dogs, some 70–88% of paraldehyde (depending on the dose) is destroyed, whereas 11–28% is eliminated *via* the lungs and only 0·1–2·5% is excreted in the urine (Levine, Gilbert and Bodansky, 1939). Destruction of paraldehyde probably takes place in the liver, for in dogs with liver damage produced by chloroform anaesthesia the pulmonary excretion is increased, although the renal excretion remains small, and the hypnotic action is prolonged (Levine *et al.*, 1939). Similar results were found in mice by Hitchcock and Nelson (1943). Normal mice excrete by the pulmonary route about 8% of orally administered paraldehyde, whereas mice in which liver injury has been produced by carbon tetrachloride, excrete as much as 26% through the lungs.

It appears probable that the fate of metabolised paraldehyde in the animal body (cf. Hitchcock and Nelson, 1943) can be represented as follows:—

$$(CH_3CHO)_3 \rightarrow CH_3CHO \rightarrow CH_3COOH \rightarrow CO_2 + H_2O$$

**Heptaldehyde** (œnanthal). Boyland (1940) was unable to detect any of the possible metabolic products (heptyl alcohol, heptoic and pimelic acids) of heptaldehyde in the urine of rabbits and rats receiving it orally and concluded that it was completely oxidised in the body. Heptaldehyde is of special interest since it has been the subject of numerous studies in relation to the chemotherapy of cancer. Natural oil of wintergreen (gaultheria oil), when fed daily to mice, has the property of delaying tumour development. The active substance occurs in the low-boiling fractions of the oil and has been identified as heptaldehyde. Whether heptaldehyde has a specific action on neoplastic cells is doubtful. It probably inhibits some animal tumours by interfering with the metabolism, since it is known that heptaldehyde causes an inhibition of glycolysis. Heptaldehyde injected into mice with spontaneous tumours has a destructive action on the kidneys and the liver, and therefore may have a general toxic action on tissues which will far outweigh tumour inhibition. It may be that it leads to a debilitated general health of the animals, thus indirectly inhibiting tumour growth as a result of impaired nutrition (see Stern and Willheim, 1943).

Boyland (1940) tested the cancer-inhibiting activity of a number of possible intermediary metabolites of heptaldehyde. Heptoic acid had no effect on either grafted or spontaneous tumours, and pimelic acid had only a slight effect. A possible $\beta$-oxidation product of pimelic acid, and consequently of heptaldehyde, is malonic acid.

$$CH_3(CH_2)_5CHO \rightarrow CH_3(CH_2)_5COOH \xrightarrow{\omega\text{-oxidation}}$$

$$COOH(CH_2)_5COOH \xrightarrow{\beta\text{-oxidation}} COOHCH_2COOH$$

This acid had definite inhibiting actions and was more active than heptaldehyde. This latter fact suggests that the effect of heptaldehyde may not be due entirely to the toxic action of the aldehyde group (Boyland, 1940).

## HALOGENATED ALDEHYDE HYDRATES

Whereas unsubstituted aldehydes are easily oxidised *in vivo*, it appears probable, from our knowledge of the metabolism of chloral hydrate, that halogen substituted aldehydes are only oxidised with difficulty. Feeding of the aldehyde hydrates, chloral, bromal and butylchloral hydrates to animals results in the excretion of conjugated glucuronic acids, and it appears that the hydrates are reduced, probably *via* the free aldehyde, to the corresponding halogenated alcohol, thus :—

$$CCl_3CH(OH)_2 \rightarrow CCl_3CHO \rightarrow CCl_3CH_2OH \rightarrow CCl_3CH_2OC_6H_9O_6$$

This mechanism, however, has been questioned by Lehmann and Knoefel (1938) on pharmacological grounds (see below).

**Chloral Hydrate.** Chloral hydrate was introduced into medicine some eighty years ago as a soporific on the supposition that it would act indirectly through the formation in the organism of chloroform. However, several investigators failed to detect chloroform in blood or expired air and the error of this supposition soon became evident.

In 1875 Mering and Musculus isolated urochloralic acid from the urine of patients treated with chloral hydrate. At that time glucuronic acid had not been discovered and therefore the constitution of urochloralic acid remained obscure for some years. In 1878 Jaffe suggested that urochloralic acid might be a derivative of his newly discovered " Glykuronsäure ", and this was confirmed by Külz (1882) and by Mering (1882).

Külz (1882) showed that urochloralic acid was a compound of trichloroethyl alcohol and glucuronic acid and the main product of chloral hydrate metabolism in man, dog and rabbit. He further showed that, unlike its precursor, urochloralic acid was devoid of hypnotic action. At first there was some doubt as to the glycosidic structure of urochloralic acid since it reduced alkaline copper reagents (i.e. Fehling's solution).[1] But there is now little doubt that its constitution is that of trichloroethyl-$\beta$-glucuronide, $CCl_3CH_2O.C_6H_9O_6$, and its reducing properties and those of urobromalic acid, $CBr_3CH_2O.C_6H_9O_6$, can be explained by the instability in the presence of strong alkali of three halogen atoms attached to the same carbon atom. Urochloralic acid is a crystalline, strongly acidic solid (m.p. 142°) which is laevorotatory in aqueous solution ($[\alpha]_D - 60°$; Na salt, $- 65{\cdot}2°$). About 50% of the chloral hydrate fed to dogs can be accounted for as this conjugate (Külz, 1882).

A similar compound was isolated by Külz from the urine of dogs receiving **butylchloral hydrate,** $CH_3CHClCCl_2CH(OH)_2$. The glucuronide, urobutylchloralic acid, was isolated as its laevorotatory, crystalline potassium salt, $C_{10}H_{15}Cl_3O_7K$. Urobutylchloralic acid is non-reducing, for the three halogen atoms are not attached to the same carbon atom as in urochloralic acid. Its structure is presumably that of 2:2:3-trichlorobutyl-1-glucuronide, $CH_3CHClCCl_2CH_2OC_6H_9O_6$.

**Bromal hydrate** is also converted *in vivo* to a conjugated glucuronic acid (Maraldi, 1903; Vitali, 1899) which is probably tribromoethylglucuronide (see tribromoethyl alcohol).

From a consideration of the pharmacodynamic properties of chloral hydrate, bromal hydrate, trichloroethyl alcohol and tribromoethyl alcohol, Lehmann and Knoefel (1938) were led to doubt whether chloral and bromal hydrates were converted through an intermediate reduction to the corresponding primary alcohols, to trichloro- and tribromo-ethylglucuronides respectively. The duration of action of chloral hydrate is briefer than that of trichloroethyl alcohol and the conversion of the latter to inactive metabolites proceeds very slowly. Lehmann and Knoefel argue that " if chloral hydrate were reduced in the organism to trichlorethanol, the duration of action of the former should be longer for an extra step is required for its detoxication. Since the effect of chloral hydrate is briefer, it seems unlikely that it forms trichlorethanol as such ". It appears probable that trichlorethanol plays no part in the hypnotic action of chloral hydrate.

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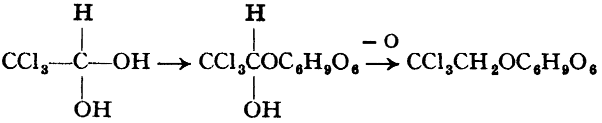
[1] Urochloralic acid does not reduce Benedict's reagent.

The cases of bromal hydrate and tribromoethyl alcohol are also of great interest. The effects of bromal hydrate are long lasting and doses of 40 mg./kg. cause death in some cases, but after at least 20 hours. At this dose level tribromoethyl alcohol has no discernible action.

The alternative suggestion put forward by Lehmann and Knoefel to account for the excretion of trihalogenated ethylglucuronides after the administration of chloral and bromal hydrates is that the hydrate combines directly with glucuronic acid, forming, presumably, an aldehyde hydrate glucuronide which is then converted in some way to the halogenated ethylglucuronide. The writer envisages the mechanism as follows :—



$$CCl_3\text{—}\overset{\displaystyle H}{\underset{\displaystyle OH}{C}}\text{—}OH \rightarrow CCl_3\overset{\displaystyle H}{\underset{\displaystyle OH}{C}}OC_6H_9O_6 \xrightarrow{-O} CCl_3CH_2OC_6H_9O_6$$

## ALIPHATIC KETONES

Few investigations have been carried out on the metabolism of aliphatic ketones in the animal body, but it appears probable that many of them are partly reduced to secondary alcohols which are then excreted in conjugation with glucuronic acid. In the case of the lower ketones a considerable amount may be excreted unchanged. **Acetone** is notoriously difficult to oxidize *in vivo*, and according to Schwarz (1898), when it is administered orally to dogs in doses of about 0·3 to 0·5 g./kg., 50–60% or more is excreted unchanged in the urine (but see dichloroacetone below). If one of the methyl groups in acetone is replaced by ethyl or *n*-propyl as in **methylethylketone** or **methylpropylketone,** less of the unchanged ketone is excreted, for only 30–33% of methylethylketone (dose, 0·3 to 0·4 g./kg.) and 21–27% of methylpropylketone (dose, 0·35 to 0·4 g./kg.) is excreted unchanged. When both methyl groups are replaced, as in the case of **diethylketone,** little or no unchanged ketone is excreted in the urine, for Schwarz (1898) recovered unchanged from dog's urine only 8·8% of diethylketone fed at a dose level of 0·33 g./kg., and none at a dose level of 0·1 g./kg.

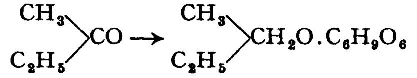
TABLE 2

*Excretion of the Lower Ketones*

| Compound | Formula | Percentage of dose excreted unchanged (dog) | Glucuronide formation (rabbit) |
|---|---|---|---|
| Acetone | $CH_3COCH_3$ | 50–60 or more | none |
| Methylethylketone | $C_2H_5COCH_3$ | 30–33 | + |
| Methylpropylketone | $C_3H_7COCH_3$ | 21–27 | + |
| Diethylketone | $C_2H_5COC_2H_5$ | 0– 9 | + |

Neubauer (1901) studied qualitatively the formation of glucuronides in the rabbit after feeding a number of ketones. He found that the administration of acetone did not result in increased glucuronic acid excretion, but that with other ketones (methylethyl, methylpropyl, methyl*iso*propyl, diethyl, methylbutyl, methyl *tertiary* butyl (pinacoline), ethylpropyl, ethyl*iso*propyl, methylhexyl) the excretion of glucuronic acid was enhanced. **Mesityl oxide** and **acetylacetone** also formed glucuronides.

In one case a glucuronide has been isolated. Saneyoshi (1911) prepared the barium salt of the glucuronide of normal secondary butyl alcohol (2-butanol glucuronide) from the urine of rabbits which had been fed methylethylketone.



$$\left.\begin{matrix}CH_3\\C_2H_5\end{matrix}\right>CO \rightarrow \left.\begin{matrix}CH_3\\C_2H_5\end{matrix}\right>CH_2O.C_6H_9O_6$$

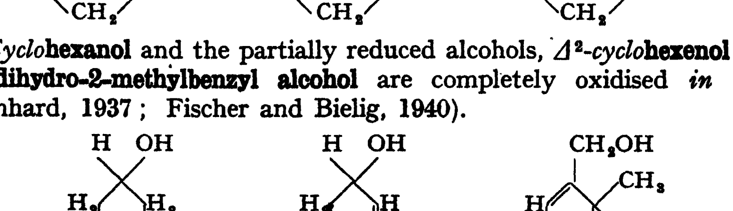
No constants for this salt were given.

Although acetone itself is not reduced *in vivo* to the corresponding alcohol, its **dichloroacetone** is reduced to dichloro*iso*propyl alcohol and excreted as dichloro*iso*propylglucuronide (Sundvik, 1886).

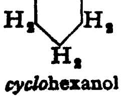
## The Metabolism of *Cyclo*hexane and its Derivatives

The dehydrogenation of saturated rings in the animal body is of considerable interest because it has been suggested that compounds such as sterols and sex hormones, which contain reduced rings, may be dehydrogenated to carcinogens. With this possibility in mind the fates of a number of *cyclo*hexane derivatives have been studied, and whilst it has been shown that their dehydrogenation *in vivo* does occur, its extent is dependent on the nature of the substituents in the *cyclo*hexane ring.

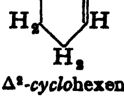
*Cyclo***hexane** itself on injection into rabbits is converted into *cyclo*-**hexanone**, and this ketone on injection undergoes ring fission with the formation of adipic acid (Filippi, 1914) :—



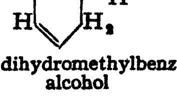
*Cyclo***hexanol** and the partially reduced alcohols, $\Delta^2$-*cyclo***hexenol** and $\Delta^{46}$**-dihydro-2-methylbenzyl alcohol** are completely oxidised *in vivo* (Bernhard, 1937 ; Fischer and Bielig, 1940).



*cyclo*hexanol



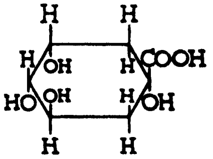
$\Delta^2$-*cyclo*hexenol



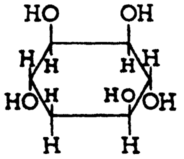
$\Delta^{4:6}$-dihydromethylbenzyl alcohol

### Polyhydroxy*cyclo*hexanes

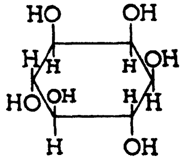
***l*-Quinic acid** (1:3:4:5-tetrahydroxy*cyclo*hexylcarboxylic acid) is found in a number of plant products such as quinine bark, coffee beans, bilberries, etc. Lautemann (1863) claimed that in man quinic acid is excreted as hippuric acid, and Schmid (1905) found that on subcutaneous injection into dogs it causes an increased excretion of ethereal sulphates. It appears from these results that quinic acid may be dehydrogenated *in vivo* to aromatic compounds. Bernhard (1937), however, denies this, for on feeding or injecting *l*-quinic acid he found no benzoic acid in the urine and at least 50% of the acid was excreted unchanged. Stadelmann (1879) found that quinic acid was also excreted unchanged by herbivorous animals.



quinic acid



quercitol



mesoinositol (according to Dangschat and Fischer, 1942)

**Quercitol** (pentahydroxy*cyclo*hexane), which is found in acorns and oak bark and is sometimes called "acorn sugar", is mainly excreted unchanged by animals (Mering, 1877; Pohl, 1896).

**Inositol** (hexahydroxy*cyclo*hexane [1]) has been found in nature in four forms. The isomeride of greatest interest is mesoinositol. It occurs in considerable quantities in plants as the calcium, magnesium and potassium salts of phytic acid—mesoinositol hexaphosphoric acid—a substance which, if present in foodstuffs in appreciable amounts, interferes with the absorption of calcium and iron in the intestine. Mesoinositol has also been identified with the plant growth-promoting substance Bios I; it has been classified as a B vitamin and is the anti-alopecia factor for mice.

When fed to man, the rabbit or the dog, only small amounts of mesoinositol are excreted unchanged in the urine. In dogs it has a strong purgative action, causing diarrhoea, and as much as 77% of the dose is excreted mainly in the faeces (Anderson and Bosworth, 1916). On injection into rabbits, however, nearly 50% may be eliminated unchanged in the urine and it appears probable that much inositol, when given by the oral route, is destroyed by bacteria in the intestine. In man, only 9% of orally administered mesoinositol is eliminated in the urine and none is found in the faeces; the fate of the other 91% is unknown (Anderson and Bosworth, 1916).

***Cyclo*hexylcarboxylic Acid.** When the *cyclo*hexane ring carries a carboxyl group attached directly to the ring, dehydrogenation to a benzene nucleus takes place and aromatic compounds are excreted (Bernhard,

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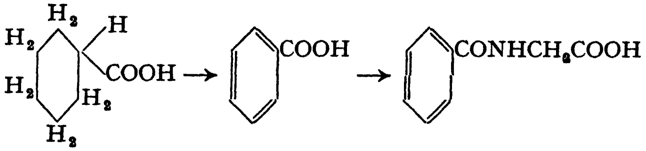
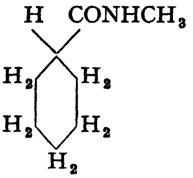
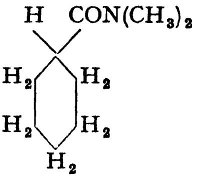
[1] Nine isomerides of hexahydroxy*cyclo*hexane are theoretically possible.

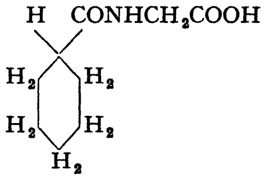
1937). Thus if **hexahydrobenzoic acid** (*cyclo*hexylcarboxylic acid) is injected into dogs, 58·9% of it is dehydrogenated to benzoic acid which appears in the urine as hippuric acid (Bernhard, 1937; cf. also Friedmann, 1911).



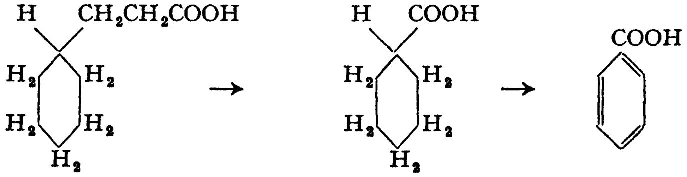
Derivatives of *cyclo*hexylcarboxylic acid in which the carboxyl group is modified, also form hippuric acid when injected into dogs. Thus 39·9% of hexahydro-N-methylbenzamide (I), 28·4% of hexahydro-N:N-dimethylbenzamide (II) and 44·5% of hexahydrohippuric acid (III) can be recovered as hippuric acid.


I


II

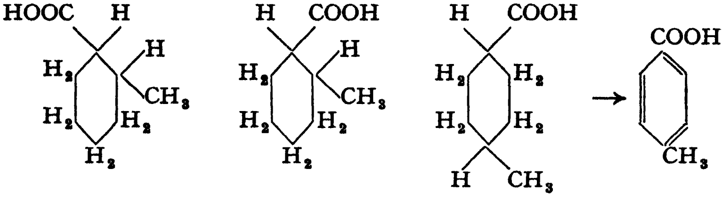

III

*Cyclo***hexylpropionic acid** is converted to hippuric acid to the extent of 44·5% and in this case a $\beta$-oxidation, with the production of *cyclo*hexylcarboxylic acid, as well as dehydrogenation, is involved.



The introduction of methyl groups into the *cyclo*hexyl derivatives has some interesting effects on the dehydrogenation. The introduction of a methyl group into the side-chain attached to the carboxyl group hinders dehydrogenation. In the case of hexahydrobenzoylsarcosine, $C_6H_{11}CO.N(CH_3)CH_2COOH$, and of hexahydrobenzoylalanine, $C_6H_{11}CONH.CH(CH_3)COOH$, dehydrogenation does not take place and these substances are mainly excreted unchanged.

When the methyl group is attached to the ring, as in the *cis* and *trans* **hexahydro-*o*-toluic acids**, dehydrogenation is again hindered, since 42% of injected *cis*-**hexa**hydro-*o*-toluic acid and 58% of *trans*-**hexa**hydro-*o*-toluic acid is excreted unchanged. The same results are obtained with **hexahydro-*m*-toluic acid,** but with **hexahydro-*p*-toluic** acid some dehydrogenation does take place and a mixture of *p*-toluic acid and the original acid is excreted.

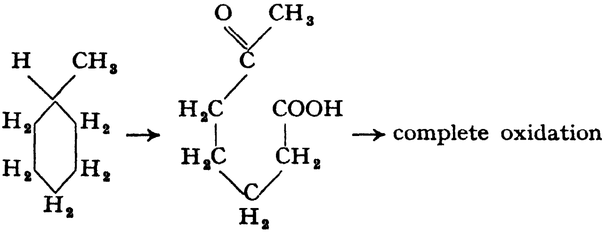


*cis*- and *trans*- hexahydro-*o*-toluic acids

hexahydro-*p*-toluic acid

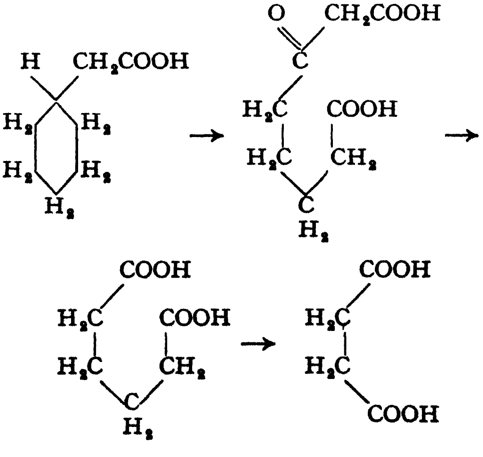
*p*-toluic acid

When the methyl group occurs alone as in **methyl*cyclo*hexane**, no products appear in the urine and the molecule is completely oxidised. This is in contrast with toluene which is oxidised *in vivo* to benzoic acid. According to Bernhard (1937), oxidation of the methyl group in methyl*cyclo*hexane probably does not occur since one could expect the resulting acid, *cyclo*hexylcarboxylic acid, to be dehydrogenated and excreted as hippuric acid. Bernhard suggests that the primary reaction in the metabolism of methyl*cyclo*hexane is opening of the ring as follows :—



*o*- and *p*-**Dimethyl*cyclo*hexane** behave similarly, being completely oxidised, for no trace of toluic acid appears in the urine.

*Cyclo***hexylacetic acid** undergoes ring fission in the body probably in a similar manner to methyl*cyclo*hexane. Succinic acid can be isolated from the urine, but no trace of phenylacetic acid is found. The steps in the conversion of *cyclo*hexylacetic acid to succinic acid are, according to Bernhard, probably as follows :—



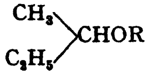
It should be noted that a ring carbon atom is here the β-carbon atom which undergoes β-oxidation (cf. *cyclo*hexylpropionic acid).

When an $NH_2$ group is attached to the *cyclo*hexane ring as in **amino-*cyclo*hexane** (hexahydroaniline), the molecule undergoes complete oxidation and no trace of either aniline or aminophenol appears in the urine.

It is clear from what has been said above that dehydrogenation of the *cyclo*hexane ring to a benzene nucleus only takes place when a COOH group or a potential COOH group is attached to the ring. A methyl group attached to the ring, particularly when in the *ortho* position to the COOH, or in some cases, in the potential carboxyl group, tends to hinder dehydrogenation. Other single substituents such as $NH_2$, OH, $CH_2COOH$ or $CH_3$ are not favourable to dehydrogenation and *cyclo*hexyl derivatives containing any of them are completely oxidised, for if dehydrogenation did take place benzene derivatives would be formed and, since these are notoriously difficult to oxidise *in vivo*, their presence could easily be detected in the urine.

## TABLE 3

*Biosynthetic Aliphatic Glucuronides*

| Compound | Formula[1] | M.P. °C. | [α]D in water | Isolated from | References |
|---|---|---|---|---|---|
| Trichloroethylglucuronide | $CCl_3CH_2OR$ | 142 | − 65·2° (Na salt)<br>− 60 (acid) | Urine of men, dogs and rabbits receiving chloral hydrate | Thierfelder (1886) ;<br>Külz (1882) |
| Tribromoethylglucuronide | $CBr_3CH_2OR$ | 145·5 | − 79·09 | Urine of rabbits receiving tribromoethyl alcohol | Endoh (1924) |
| 2:2:3-Trichlorobutylglucuronide | $CH_3CHClCCl_2CH_2OR$ | — | Laevo (only K salt known) | Urine of dogs receiving butyl-chloral hydrate | Külz (1882) |
| Methylethylmethyl- or secondary butyl-glucuronide |  $\begin{matrix} CH_3 \\ C_2H_5 \end{matrix}\!\!>\!CHOR$ | — | No constants determined, Ba salt only known | Urine of rabbits receiving methyl-ethyl ketone | Saneyoshi (1911) |
| Trimethylmethyl- or tertiary butylglucuronide | $(CH_3)_3COR$ | — | − 28·5 (K salt) | Urine of rabbits receiving tertiary butyl alcohol | Thierfelder and Mering (1885) |
| Dimethylethyl- or tertiary amylglucuronide | $(CH_3)_2C_2H_5COR$ | — | − 31·1 (K salt) | Urine of rabbits receiving tertiary amyl alcohol | ditto |

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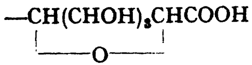
[1] R =  $—\underbrace{CH(CHOH)_3CH}_{O}COOH$

## *Chapter Three*

# The Metabolism of Aromatic Hydrocarbons

### BENZENE

THE fate of benzene in the animal body has been the subject of numerous investigations, and although most of its metabolites seem to have been identified, the order in which they appear is by no means certain. The quantitative aspects of the problem remain to be studied in detail.

Very little benzene is excreted unchanged in the urine except when large doses are administered. Drummond and Finar (1938), for example, found that no free benzene was excreted in the urine of rabbits receiving less than 3 c.c. (*ca.* 2·6 g.), but with larger doses the free hydrocarbon could be detected. Much benzene, however, may be eliminated unchanged by the lungs. Nencki and Sieber (1883) estimated that about one-third of the quantity absorbed left the body through the lungs, whereas Lehmann, Gundermann and Kleiner (1910) put the figure as 40–45% of that absorbed.

The main products of benzene metabolism in the animal are phenols (phenol, catechol and quinol) and their conjugates, the glucuronides and ethereal sulphates. Small amounts of muconic acid and of phenylmercapturic acid are also formed.

**Oxidation to Phenol.** The oxidation of benzene to phenol in the animal body was discovered by Schultzen and Naunyn in 1867. This observation was confirmed by Munk (1876), who showed that the amount of phenol excreted increased with the dose of benzene. The latter point was important since normal urine contains phenol and the isolation of phenol from urine would not necessarily have been a proof of its formation from benzene unless it could be shown to increase after the administration of benzene. Munk distilled " benzene " urine with sulphuric acid and obtained tribromophenol from the distillate after treatment with bromine water. The early work of Nencki and Sieber (1883) indicated that about a quarter or a third of the absorbed benzene was oxidised in rabbits, whereas Schmiedeberg (1881) found that of 21 g. of benzene administered to a dog, 8·5% was excreted as phenols (1·884 g. of phenol and 0·445 g. of dihydroxybenzenes). Boruttau and Stadelmann (1914) found about a quarter to a third to be oxidised to phenols in man and about one-tenth in the dog. More recent work by Braunstein, Parschin and Chalisowa (1931) on the rabbit confirms the estimate of Nencki and Sieber, for they find that about 25% of a single injection of benzene (1 g. to rabbits of 1·5–1·9 kg. body weight) is excreted by rabbits as phenol and its conjugates. The amount of conjugated phenol excreted is, however, variable and depends on the nature of the diet, or rather upon the extent of diuresis resulting from the diet (Braunstein and Parschin, 1931); with repeated injection of benzene, the amount is less than for a single dose. These results are shown in the following table.

TABLE 4

*Phenol excreted by the Rabbit after Single and Repeated Injection of Benzene* (*Braunstein et al.*, 1931)

SINGLE INJECTION OF BENZENE

| Diet [1] | Excretion of injected benzene | | Combined phenol as % of total phenol |
|---|---|---|---|
| | Total phenol % | Combined phenol % | |
| Acid (28) [2] | 24·8 | 9·4 | 37·9 |
| Basic (28) | 28·1 | 5·0 | 17·8 |
| Mixed (21) | 24·8 | 7·5 | 30·2 |

[1] *Acid diet:* 200 g. oats, 10 g. cabbage, 5 g. hay. *Basic diet:* 200 g. potatoes, 100 g. cabbage, 5 g. hay.

[2] Number of experiments.

REPEATED INJECTION OF BENZENE

| Diet | Excretion of injected benzene | | Combined phenol as % of total phenol |
|---|---|---|---|
| | Total phenol | Combined phenol | |
| Acid (23) | 15·0 | 6·0 | 40·0 |
| Basic (24) | 23·1 | 2·0 | 8·7 |
| Mixed (13) | 18·5 | 4·6 | 24·9 |

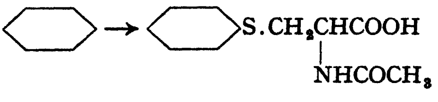
In the experiments of Braunstein *et al.* (1931) phenol was estimated by a method based on the Folin-Denis procedure for the determination of phenol in urine, and it is probable that their figures for phenol include benzene oxidised to catechol and quinol.

Benzene not only forms phenol in the body but also the dihydroxybenzenes, catechol and quinol. Nencki and Giacosa (1880) were able to isolate catechol in a crystalline state from the urines of both dogs and humans after large doses of benzene (5–6 g. per day). Quinol was also present in the human urines, but its isolation in a pure state was not achieved. Nencki and Giacosa (1880) state that they could not detect quinol in the dog urine from which they isolated catechol. Methods for the determination of phenol, catechol and quinol have recently been worked out by Baernstein (1945), who found that on repeated daily injections of 2 c.c. of benzene into rabbits, the excretion of all three phenols gradually increased, reaching a peak on the 9th day and then falling to almost normal values on the 15th day, despite the fact that benzene was still being injected. The drop in the excretion of the phenols was followed rapidly by the death of the animal.

The main site of the oxidation of benzene is apparently the liver. Tschernikow, Gadaskin and Gurewitsch (1930) showed that the isolated livers of the cat, the rabbit and the frog could oxidise benzene to phenol and that the livers of benzene-poisoned animals contained both free and conjugated phenol. In the frog, at least, the liver is not the only organ which converts benzene to phenol, and it appears probable that warm-blooded animals also have extra-hepatic sites for the oxidation of benzene (Tschernikow *et al.*, 1930).

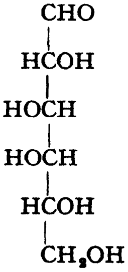
The formation of phenols by the oxidation of benzene results in the enhanced excretion of ethereal sulphate and of conjugated glucuronic acid. These conjugates have not been isolated from " benzene urine ", but it is probable that the main conjugates are phenylglucuronide and phenylsulphuric acid.

**The Formation of Phenylmercapturic Acid.** Fractions from the urine of rabbits receiving benzene give, on treatment with hydrochloric acid, small amounts of thiophenol which may be derived from phenylmercapturic acid (Drummond and Finar, 1938). More recent work, however, indicates that the rabbit probably does not excrete phenylmercapturic acid (Witter, 1945). The isolation of phenylmercapturic acid from benzene urine defied the efforts of many workers, no doubt because of its instability in slightly alkaline media and the fact that the amount of benzene converted to this conjugate is very small. Its successful isolation was eventually achieved by Zbarsky and Young (1943*a*), who first synthesised it and then isolated it from rat urine after feeding phenyl-*l*-cysteine. This latter compound when fed to rats is acetylated to phenylmercapturic acid and as much as 38% of the administered phenylcysteine can be isolated as crystalline *l*-phenylmercapturic acid (Zbarsky and Young, 1943*b*). From the urine of rats receiving benzene in corn oil orally (to delay absorption) up to 0·37% of the benzene fed can be isolated as crystalline *l*-phenylmercapturic acid, whereas if the benzene is fed without a diluent, the maximum yield of crystalline material is only 0·11% (Zbarsky and Young, 1943*a*).



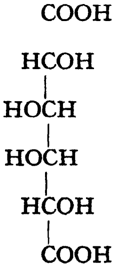
Phenylmercapturic acid is, however, only a minor metabolite of benzene.

**The Formation of Muconic Acid.** There is now little doubt that a muconic acid is produced during the metabolism of benzene, although since Jaffe in 1909 isolated it from the urine of dogs and rabbits receiving benzene, there has been much controversy regarding its occurrence. It has also been isolated from human urine, for Fuchs and Soos (1916) obtained it in a 0·11% yield from the urine of patients treated for leukaemia with benzene. Jaffe was of the opinion that something like 20–30% of the benzene administered to the animal formed muconic acid in the tissues. He isolated as muconic acid approximately 0·3% of the benzene administered, and the rest of the acid formed from benzene was considered to be quickly metabolised since only about 1% of injected muconic acid could be recovered from the urine. Yields of 3·7% were recorded by Thierfelder and Klenk (1924), 0·4% by Drummond and Finar (1938) and 0·5% by Bernhard and Gressly (1941). On the other hand, both Neumaerker (1923) and Mori (1918) failed to detect muconic acid in the urine of rabbits after the administration of benzene in high doses. Variable results were also obtained on the recovery of muconic acid from urine after it had been injected into animals. Jaffe recovered less than 1%, Bernhard and Gressly (1941) recovered none at all, whereas Neumaerker (1923), Mori (1918) and Drummond and Finar (1938) recorded recoveries of 53, 73 and 56% respectively. These conflicting results led to the suspicion that muconic acid might not arise as a direct metabolite of benzene, but rather as a product resulting from disturbances of normal metabolism due to benzene. The issue became more confused when it was pointed out by Böeseken (Böeseken and Sloof, 1929 ; Böeseken and Engleberts, 1932) that whereas *trans-trans*-muconic acid had been isolated from benzene urine, one would expect the *cis-cis* acid to be formed, for the *cis-cis* acid is produced by the oxidation of phenol with peracetic acid at room temperature (Böeseken and Kerkhoven, 1932). The *trans-trans* acid is more likely to be a product of the oxidation and dehydration of galactose, since the spacing of the atoms in this sugar and in mucic acid renders the formation of the *trans-trans*-muconic acid possible.



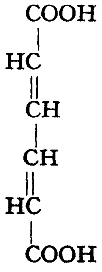
$$\begin{array}{c}CHO\\|\\HCOH\\|\\HOCH\\|\\HOCH\\|\\HCOH\\|\\CH_2OH\end{array}$$

galactose



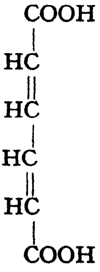
$$\begin{array}{c}COOH\\|\\HCOH\\|\\HOCH\\|\\HOCH\\|\\HCOH\\|\\COOH\end{array}$$

mucic acid



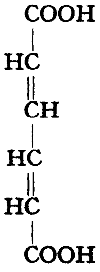
$$\begin{array}{c}COOH\\|\\HC\\\|\\CH\\|\\CH\\\|\\HC\\|\\COOH\end{array}$$

*trans-trans-*
muconic acid
m.p. 301°
(decomp.)



$$\begin{array}{c}COOH\\|\\HC\\\|\\HC\\|\\HC\\\|\\HC\\|\\COOH\end{array}$$

*cis-cis-*
muconic acid
m.p. 195°

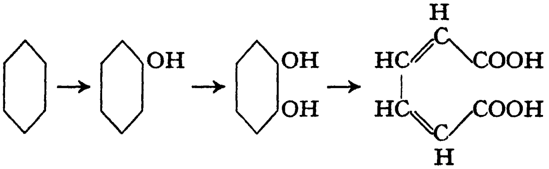


$$\begin{array}{c}COOH\\|\\HC\\\|\\CH\\|\\HC\\\|\\HC\\|\\COOH\end{array}$$

*cis-trans-*
muconic acid
(unknown)

The investigations of Drummond and Finar (1938) and of Bernhard and Gressly (1941) have thrown new light on the problem. The latter workers showed that on injecting benzene containing deuterium into rabbits, muconic acid containing deuterium is excreted. Seventeen rabbits were injected with 1 to 1·5 c.c. of benzene containing 10·7 atoms % D, and in one case muconic acid containing 7·21 atoms % D was isolated. These experiments provide direct evidence of the formation of muconic acid from benzene. The question of the isolation of the *trans-trans* form instead of the *cis-cis* form was studied by Drummond and Finar. In the first place they failed to recover any *cis-cis* acid from rabbits receiving benzene, the product was always the *trans-trans* acid. *Cis-cis* acid added to normal rabbit urine could be recovered to the extent of about 56%, whilst up to 84% of the *trans-trans* acid could be recovered. When, however, the *cis-cis* acid was added to " benzene " urine, which is rich in phenolic substances, the acid recovered was not the *cis-cis* but the *trans-trans* acid. But, by careful attention to detail and avoidance of heat during the extraction, the added *cis-cis* acid could be recovered unchanged from " benzene " urine in the presence of *trans-trans* acid. There was, therefore, an indication that " benzene " urine contained some substance or substances which might, under suitable conditions, lead to the conversion of the *cis-cis* to the *trans-trans* acid. On injecting sodium *cis-cis*-muconate with benzene into rabbits it was found that more of the *trans-trans* acid was isolated than if benzene alone had been injected. Drummond and Finar therefore came to the conclusion that, although their evidence is not unequivocal, the *cis-cis* acid is converted into the *trans-trans* acid during the metabolism of benzene. When the animal is not metabolising benzene, the *cis-cis* acid is not transformed to the *trans-trans* form. No explanation of this curious change is as yet available.

The question may now be asked : At which stage in the metabolism of benzene does opening of the ring occur ? The answer to this question can only be speculative, on our present knowledge. One possibility is that it is formed from catechol thus :—



This mechanism was considered by Drummond and Finar but they were unable to detect muconic acid in the urines of rabbits receiving the maximum non-lethal doses of both catechol and phenol. In all probability these doses were too small (the largest dose of phenol used was 0·25 g.), especially if one bears in mind that at least 3 c.c. of benzene is necessary to produce isolable muconic acid.[1]

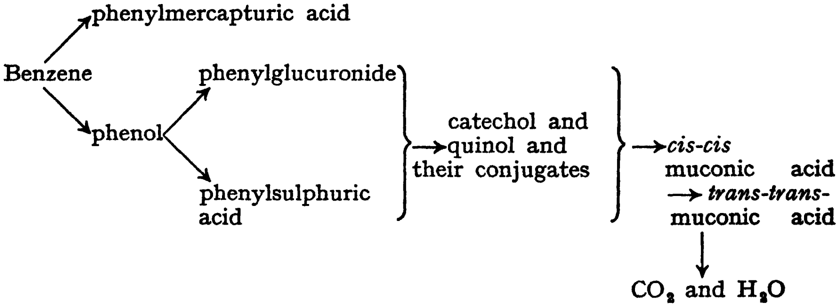
---

[1] *Trans-trans*-muconic acid is also excreted in small amounts (0·1–0·2%) when sorbic acid is fed to rabbits (Kuhn, Köhler and Köhler, 1937). The yield is increased to 0·5% if ethyl sorbate is fed, but none at all is obtained with methyl sorbate. If, however, the carboxyl group of sorbic acid is modified by forming the amide, methylamide or anilide, the yield of muconic acid derivatives excreted in the urine is considerably increased, for sorbamide fed to rabbits yields 32% of *trans-trans*-muconic acid mono-amide ; the methylamide and anilide of sorbic acid yield 44% and 36% of the methylamide and anilide of *trans-trans*-muconic acid respectively.

$CH_3CH{:}CH.CH{:}CHCOOH \longrightarrow COOHCH{:}CH.CH{:}CHCOOH$ (0·1–0·2% yield)
$CH_3CH{:}CH.CH{:}CHCOOC_2H_5 \longrightarrow COOHCH{:}CH.CH{:}CHCOOH$ (0·5% yield)
$CH_3CH{:}CH.CH{:}CHCONH_2 \longrightarrow COOHCH{:}CH.CH{:}CHCONH_2$ (32% yield)

The presence of the amide group in sorbic acid apparently tends to hinder the

On the available evidence, the fate of benzene in the animal body can be represented as follows:—



It is clear from the above tentative scheme that much remains to be done to fit each compound into the chain of reactions leading from benzene to carbon dioxide and water. No investigations as to the nature of the metabolites of muconic acid have been carried out, but it appears probable that benzene eventually does get oxidised *via* muconic acid to $CO_2$ and water.

The conversion of benzene *in vivo* to phenylsulphuric acid has application in detecting exposure to benzene vapour. It is well known that an increase in ethereal sulphate excretion takes place at the expense of the inorganic sulphate fraction of urine. According to Yant, Schrenk, Sayers, Horvath and Reinhart (1936) a marked decrease in the percentage of inorganic sulphate in the urine of workers exposed to benzene occurs long before symptoms of anaemia, etc., become evident. Urinary sulphate analysis is useful in indicating the relative degree of exposure to benzene, but it does not indicate the effect of the exposure on the worker (Jephcott and Bulmer, 1939). The latter investigators found, in 68 workers exposed to benzene, 30% to have subnormal sulphate ratios and 21% excreted more than 0·4 g./litre of ethereal sulphate.

## TOLUENE AND OTHER METHYL SUBSTITUTED BENZENES

As early as 1867 it was shown by Schultzen and Naunyn that a methyl group attached to an aromatic ring could be oxidised *in vivo* to a carboxyl group. **Toluene** administered to animals is converted mainly to benzoic acid which in turn is excreted in the urine as hippuric acid. The extent to which this oxidation takes place was studied by Epstein and Braunstein (1931) who showed that in the rabbit up to 70% of a dose of injected toluene is oxidised to benzoic acid (see Table 5). Knoop and Gehrke (1925) showed that 7 g. of toluene fed to a dog resulted in the excretion of 12 g. of hippuric acid; this accounts for 80–90% of the toluene fed.

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β-oxidation process which would result in the complete oxidation of muconic acid *in vivo*. A similar effect is observed with β-methylsorbamide which yields as much as 62% of the amide of β-methylmuconic acid

$$CH_3CH{:}CH.C(CH_3){:}CHCONH_2 \rightarrow COOHCH{:}CH.C(CH_3){:}CHCONH_2.$$

TABLE 5

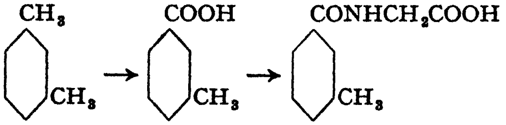
*The Oxidation of Toluene to Benzoic Acid in the Rabbit*

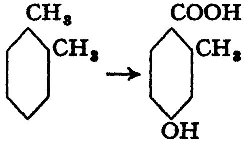
TOLUENE OXIDISED TO BENZOIC ACID

| Diet | 0·5 g. dose | 1·0 g. dose |
|---|---|---|
| Basic | 56·2% | 70·2% |
| Acid | 53·2% | 63·1% |

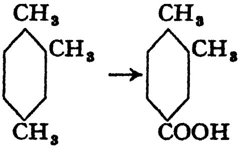
Regarding the fate in the rabbit of the other 10–30% there is no evidence. Oxidation of the ring to a phenol probably does not take place, for toluene does not cause increased excretion of ethereal sulphate (Baumann and Herter, 1877).

When more than one methyl group is attached to the ring, only one is oxidised to carboxyl, any others survive passage through the organism. ***m*-Xylene** is converted in the dog and the guinea pig to *m*-toluic acid which appears in the urine as *m*-toluric acid (Schultzen and Naunyn, 1867; Filippi, 1914); by comparison its isomeride *o*-**xylene** is almost completely oxidised in the guinea-pig to hydroxy-*o*-toluic acid (Filippi, 1914):—

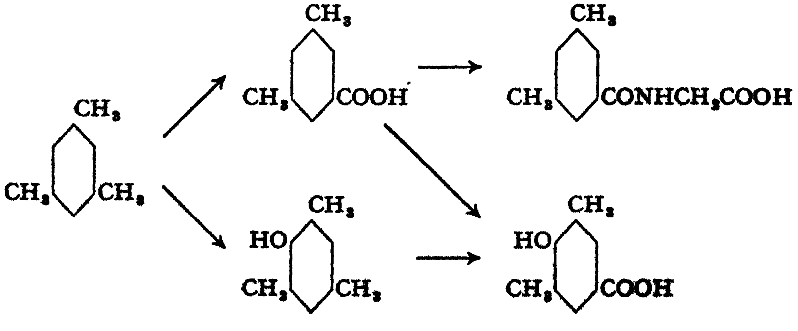




***Pseudocumene*** (1:2:4-trimethylbenzene) is oxidised to xylic acid (Jacobsen, 1879):—



**Mesitylene** (1:3:5-trimethylbenzene) is oxidised in the dog and the guinea-pig to mesitylenic acid, which is excreted partly free and partly combined with glycine (von Nencki, 1873; Filippi, 1914). According to Curci (1894), mesitol and *p*-hydroxymesitylenic acid are also excreted. Mesitylene may result in the excretion of at least four metabolites, thus:—



## HIGHER HOMOLOGUES OF BENZENE

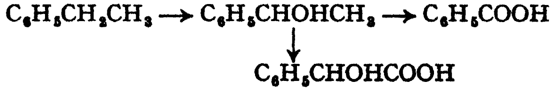
With homologues of benzene higher in the series than toluene, the nature of the oxidation products depends on the structure of the side chain. Although the amount of information available is somewhat scanty, there is sufficient to make the following tentative generalisations :—

(1) When the side-chain is a normal alkyl group, e.g. methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, bio-oxidation of the side-chain to —COOH or $—CH_2COOH$ takes place and no oxidation occurs in the ring. Aromatic acids, their glycine conjugates and, possibly, intermediate compounds in the oxidation of the side-chain are excreted.

(2) In the absence of a side-chain (i.e. in benzene), or if the side-chain is branched (e.g. *iso*propyl), oxidation takes place in the ring with the production of a phenol and there is increased excretion of ethereal sulphates. The branched side-chain is usually unattacked.

(3) If a methyl group occurs together with a branched side-chain, then the methyl group is oxidised to carboxyl, the ring and the side-chain being unchanged.

**Ethylbenzene.** Nencki and Giacosa (1880) showed that this hydrocarbon is oxidised in dogs to benzoic acid and excreted as hippuric acid, whereas Neubauer (1901) showed that it formed methylphenylcarbinol in the rabbit. A more thorough investigation by Thierfelder and Daiber (1923) showed that, in addition to benzoic acid and methylphenylcarbinol, small amounts of mandelic acid are formed in rabbits. The carbinol is excreted combined with glucuronic acid and the conjugate is identical with that produced when acetophenone is metabolised in the animal body (see p. 89). The probable absence of oxidation of the ring was shown by Nencki and Giacosa, who found that the urine excreted after administration of ethylbenzene had a normal ethereal sulphate content. Omitting conjugated products, the oxidation of ethylbenzene may take the following course :—



$$\begin{array}{ccccc} C_6H_5CH_2CH_3 & \longrightarrow & C_6H_5CHOHCH_3 & \longrightarrow & C_6H_5COOH \\ & & \downarrow & & \\ & & C_6H_5CHOHCOOH & & \end{array}$$

Further investigation is necessary before such a simple scheme as the above can be accepted as correct. However, it is almost certain that mandelic acid is not an intermediate in the formation of benzoic acid (see p. 114), since it is mainly excreted unchanged when administered to an animal. It is interesting to note that no mention has been made as to whether phenylacetic acid is or is not excreted after the administration of ethylbenzene.

***n*-Propylbenzene** has been shown to form hippuric acid in dogs (Nencki and Giacosa, 1880; Thierfelder and Klenk, 1924).

$$C_6H_5CH_2CH_2CH_3 \longrightarrow C_6H_5COOH \longrightarrow C_6H_5CONHCH_2COOH$$

***n*-Butylbenzene,** injected into dogs, is converted into phenylacetic acid (Thierfelder and Klenk, 1924) as follows :—

$$C_6H_5CH_2\overset{*}{C}H_2CH_2CH_3 \longrightarrow C_6H_5CH_2COOH$$

whereas ***n*-amylbenzene** forms benzoic acid :—

$$C_6H_5\overset{*}{C}H_2CH_2\overset{*}{C}H_2CH_2CH_3 \longrightarrow C_6H_5COOH$$

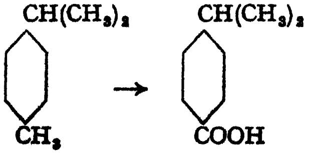
(* $\beta$ carbon atom, see below.)

We have, therefore, a complete series of benzene hydrocarbons with side-chains from the methyl to the *n*-amyl. After oxidation of the terminal $CH_3$, benzoic acid could be expected, and is formed from toluene, *n*-propyl and *n*-amylbenzenes according to the theory of $\beta$-oxidation. From ethyl- and *n*-butylbenzene, phenylacetic acid could be expected, but its formation has been recorded only in the case of the butyl derivative, the corresponding hydroxy acid, mandelic acid, and benzoic acid being excreted after injection of the ethyl derivative.

***iso*Propylbenzene** (Cumene). Unlike the *n*-propyl derivative, this hydrocarbon does not result in the excretion of hippuric acid or any other aromatic acid, rather does it cause an enhanced excretion of ethereal sulphates, thus indicating the production of phenolic substances. Nencki and Giacosa (1880) failed to isolate and characterise the phenols.

***iso*Butylbenzenes.** Two of these hydrocarbons, **dimethylbenzylmethane,** $C_6H_5CH_2CH(CH_3)_2$, and **methylethylphenylmethane,** $C_6H_5CH(CH_3)C_2H_5$, were fed to dogs by Nencki and Giacosa (1880). The results obtained were similar to those obtained with *iso*propylbenzene, in that no aromatic acid was excreted and phenols were formed as indicated by the increase of ethereal sulphates in the urine.

***p*-Cymene** (p-*iso*propyltoluene). Here we have an example of a hydrocarbon with a methyl and an *iso*propyl group attached to the same ring. If fed to dogs or sheep, the hydrocarbon is converted into cumic acid (Nencki and Giacosa, 1880 ; Harvey, 1942 ; cf. also L. von Nencki, 1873, and Ziegler, 1873). The *iso*propyl side-chain resists oxidation and since no phenol is formed, it seems that the ring also resists oxidation. The methyl side-chain, however, forms a carboxyl group as in the case of toluene.



## POLYCYCLIC HYDROCARBONS CONTAINING CONDENSED BENZENE RINGS

The fact that certain polycyclic aromatic hydrocarbons are concerned in producing pathological changes in animals has stimulated an interest in their metabolism in the organism. Thus naphthalene, when administered in repeated doses to rabbits, produces an opacity of the crystalline lens of the eye and there is a close histological resemblance between this condition and human senile cataract. A whole series of polycyclic hydrocarbons are known which produce cancer in various species. These, when administered to animals, may be converted into relatively harmless compounds by one or other of the " detoxication mechanisms " of the body; on the other hand, it is also possible that they may be converted into more active pathogenic substances.

In general, the polycyclic hydrocarbons undergo in the body the following changes :—

(1) Oxidation with the formation of (*a*) phenolic substances which are usually monohydroxy compounds (although occasionally dihydroxy compounds may be formed) and (*b*) quinones. The positions attacked metabolically are, in general, different from those which are known to be the most reactive in chemical substitutions *in vitro* (see Berenblum and Schoental, 1945), a fact which may indicate that biological oxidation of polycyclic hydrocarbons is not a simple substitution process.

(2) Reduction of the ring may occur in some cases, producing partly reduced aromatic rings.

**Naphthalene.** The products of napthalene metabolism in animals are naphthylglucuronide, naphthyl ethereal sulphate, naphthylmercapturic acid and a substance giving free naphthalene on distilling acidified " naphthalene urine " (Baumann and Herter, 1877 ; Bourne and Young, 1934 ; Stekol, 1937).

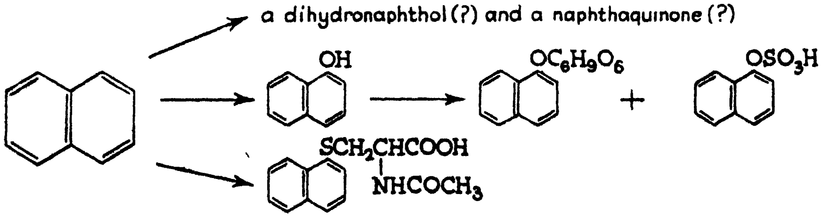
Several authors have shown that the excretion of glucuronic acid increases after the administration of naphthalene to dogs and rabbits (e.g. Lesnik and Nencki, 1886 ; Lesnik, 1888 ; Bourne and Young, 1934). Naphthalene in man and dogs is apparently oxidised at the α-position, forming α-naphthol which is excreted partly combined with glucuronic acid and partly as an ethereal sulphate (Bourne and Young, 1933). α-Naphthylglucuronide has been isolated in small amounts from the urine of a dog which had received 12 g. of naphthalene (Lesnik, 1888 ; but compare Umezawa and Masamune, 1938).

That a dihydronaphthol might exist in " naphthalene urine " has been suggested by Bourne and Young. Several workers have shown that heating " naphthalene urine " with dilute acid results in the separation of free naphthalene, and Baumann and Herter (1877) were led to think that some naphthalene was excreted unchanged. However, Bourne and Young have shown that the faintly alkaline " naphthalene urine " from rabbits does not yield free naphthalene on boiling, but only does so after acidification with hydrochloric acid. This observation could be explained by the presence in the urine of some soluble compound which is rapidly converted to naphthalene by mineral acids. Bourne and Young (1933, 1934) suggest that this compound might be 1:2-dihydro-β-naphthol. This suggestion is not unreasonable since a similar compound has actually been isolated from urine after administration of anthracene. In the rabbit a considerable proportion of administered naphthalene may be excreted as this compound (Bourne and Young, 1933).

Rabbits receiving naphthalene excrete a considerable amount of *l*-α-naphthylmercapturic acid [m.p. 170–171°, $[\alpha]_D - 25°$ in alcohol] (Bourne and Young, 1934; Ing, Bourne and Young, 1934; Stekol, 1935). This compound is of interest since it contains cysteine. It is possible that the cataract which naphthalene produces in rabbits is due to deprivation of the lens of cysteine which is used up in the detoxication of naphthalene. The protein of the lens, β-crystallin, is rich in cysteine. Glutathione and possibly free cysteine are present in the lens in relatively large amounts and it is thought that the lens, being an avascular tissue and depending on the aqueous humour for its oxygen supply, has a specialised oxidation mechanism in which cysteine-like substances are important. A reduction in the body's stores of cysteine as the result of a detoxication process involving a substance such as naphthalene might interfere with the metabolism of the lens by depriving it of cysteine and, consequently, changes resulting in cataract are induced (Bourne and Young, 1934). Cysteine is also necessary for growth in young animals and it has been shown by Stekol that young rats fed naphthalene fail to grow normally since cysteine for detoxication apparently takes precedence over that required for growth (Stekol, 1937).

Penzoldt (1886) has claimed that traces of α-naphthaquinone occur in " naphthalene urine ", but this claim has neither been confirmed nor denied by later workers. " Naphthalene urine " may also contain dihydroxynaphthalenes (cf. benzene) (Lesnik and Nencki, 1886).

The fate of naphthalene[1] in the animal body can therefore be represented approximately as follows :—



## HYDROGENATED NAPHTHALENES

**Tetrahydronaphthalene** (tetralin). Pohl and Rawicz (1919) found in rabbits, dogs and humans receiving tetralin that tetralylglucuronic acid, a dihydronaphthalene of unknown constitution and naphthalene were present in the urine. According to Röckemann (1922) the glucuronide found in dog urine is not the same as that found in rabbit urine (cf. anthracene). Rabbits probably excrete *ac*-β-tetralylglucuronide, an uncrystallisable syrup which gives dextrorotatory *ac*-β-tetralol on hydrolysis; β-tetralol is only converted with difficulty into dihydronaphthalene. From the crude glucuronide obtained from dogs, *ac*-α-tetralol, a substance which is easily converted into dihydronaphthalene,

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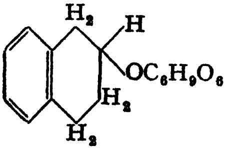
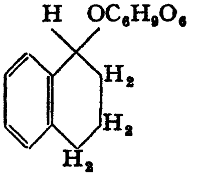
[1] Young (1946) has recently isolated 1:2-dihydronaphthalene-1:2-diol from rabbit urine.



*ac*-β-tetralylglucuronide



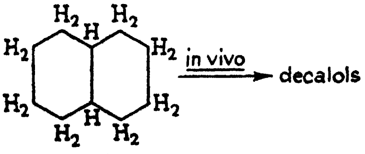
*ac*-α-tetralylglucuronide

was isolated. Bernhard (1939) was unable to isolate the glucuronide after feeding 30 g. tetralin to dogs, but on ether extraction of the urine, followed by distillation in vacuum, he obtained an oily mixture of di- and tetrahydronaphthols.

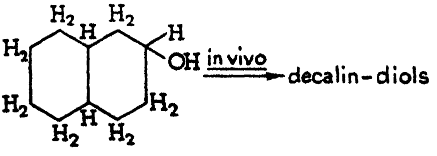
It is not clear whether some of the reported products of tetralin metabolism are artefacts of the isolation procedures or naturally formed substances. However, it is clear that, in tetralin, the reduced ring is oxidised, the aromatic ring being unaffected *in vivo*.

**Decahydronaphthalene** (decalin). From the urine of dogs receiving a *cis* and *trans* mixture of decalin orally, Bernhard (1939) was able to isolate from the urine a decahydronaphthol corresponding to 37·7% of the hydrocarbon administered. Very little glucuronide formation was detected and Bernhard was of the opinion that the greater part of the administered decalin was completely burnt in the animal. The decahydronaphthol obtained was a liquid giving an intense blue-violet colour with $FeCl_3$ in $H_2SO_4$. (Pure decahydro-β-naphthol occurs in *cis* and *trans* modifications ; the *cis* modification occurs in two forms, m.p. 105° and 17°, and the *trans* forms have m.p. 75° and 53°.)

**β-Decalol** (a *cis-trans* mixture, m.p. 63·5–72·5°) was also fed to dogs and 33% was excreted unchanged (Bernhard, 1939). A small quantity of crystalline decalindiol was also isolated, but it could not be identified with any of the known decahydrodihydroxynaphthalenes. The question of the identification of these hydroxy compounds is, of course, complicated by the occurrence of *cis* and *trans* isomerism.



decalin

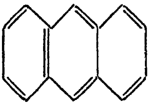


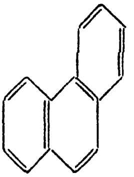
β-decalol

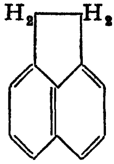
## THREE-RINGED HYDROCARBONS

Of this group, three hydrocarbons, namely, anthracene, phenanthrene and acenaphthene, have been studied.

**Phenanthrene.** Our knowledge of the metabolism of this hydrocarbon is very unsatisfactory. As the basic nucleus of many compounds of biological importance it is surprising that so little is known regarding the fate of phenanthrene itself in the animal body. It is, however, extremely difficult to obtain phenanthrene in a really pure condition.


anthracene


phenanthrene



acenaphthene

Bergel and Pschorr (1903) have shown that when phenanthrene is fed to rabbits, the resulting urine is strongly laevorotatory. These workers claimed that phenanthrene was oxidised to a phenanthrol which was excreted as a phenanthrylglucuronide. They calculated from observations of the rotation of " phenanthrene urine " that the specific rotation of this glucuronide should be about $[\alpha]_D - 35°$. The compound was present in the basic lead precipitate from the urine and this precipitate on distillation with zinc dust gave phenanthrene. In rats it forms an ethereal sulphate (Elson, Goulden and Warren, 1945).

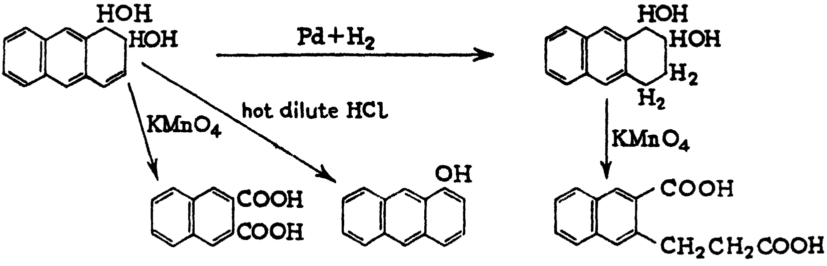
**Anthracene.** The metabolism of anthracene has been thoroughly investigated by Boyland and Levi (1935, 1936). They showed that the probable urinary products of anthracene metabolism are 1:2-dihydroxy-1:2-dihydroanthracene and its glucuronide, a hydroxydihydroanthracene, 1-anthrylmercapturic acid and, possibly, 9:10-anthraquinone. In rats a large proportion of anthracene may be excreted in the faeces (Chang, 1943). This is also true for the more complex polycyclic hydrocarbons but not for the simpler ones (see Table 6). It forms no ethereal sulphate in rats (Elson *et al.*, 1945).

TABLE 6

*The Faecal Excretion of Polycyclic Hydrocarbons in the Rat* (*after Chang,* 1943)

| Compound | Percentage of dose found in the faeces | |
|---|---|---|
| | 200 mg. hydrocarbon fed by stomach tube with starch solution | Hydrocarbon incorporated in the food; 1% of diet |
| Naphthalene | 0 | 0 |
| Phenanthrene | 7 | 5 |
| Acenaphthene | 10 | 6 |
| 3:4-Benzpyrene | 57 | 42 |
| Methylcholanthrene | 69 | 67 |
| Chrysene | 85 | 79 |
| Anthracene | 69 | 83 |
| 1:2:5:6-Dibenzanthracene | 97 | 90 |

*Dihydroxydihydroanthracene.* Rats and rabbits on a diet containing anthracene excrete different stereoisomerides of 1:2-dihydroxy-1:2-dihydroanthracene. The compound obtained from rat urine by ether extraction at neutral *p*H forms white prisms, m.p. 160–161°, $[\alpha]_D - 100°$ (in dioxan), whilst that from rabbit urine forms plates, m.p. 184° and $[\alpha]_D + 16°$ (in dioxan). The optical activity indicates that the carbon atoms bearing the OH groups are the reduced ones. Both compounds give the same naphthalene dicarboxylic acid, i.e. the 2:3-dicarboxylic acid, on oxidation with $KMnO_4$. They both give α-anthrol on heating with dilute acid. Hydrogenation with palladium and hydrogen followed by permanganate oxidation of the resulting dihydroxytetrahydroanthracene results in the formation of naphthalene-2-carboxy-3-propionic acid. These reactions show that the dihydroxy compound is probably 1:2-dihydroxy-1:2-dihydroanthracene.



The yield of the compound corresponds to about 0·5% of the anthracene fed.

*Dihydroxydihydroanthraceneglucuronic Acids.* Two of these glucuronides were isolated by Boyland and Levi (1936). The first [m.p. 197° and $[\alpha]_D + 197°$ (in dioxan) ] was isolated by amyl alcohol extraction of rabbit urine, whereas the second [m.p. 199–200° and $[\alpha]_D - 114°$ (in dioxan) ] occurred in rat urine after feeding anthracene. Rabbits excrete considerably more of the conjugated glucuronide than do rats. The glucuronide from rabbits' urine was shown to be 1:2-dihydroxy-1:2-dihydroanthracene-1-glucuronic acid by the following series of reactions :—

(1) Oxidation of the glucuronide with alkaline $KMnO_4$ gives naphthalene-2:3-dicarboxylic acid.

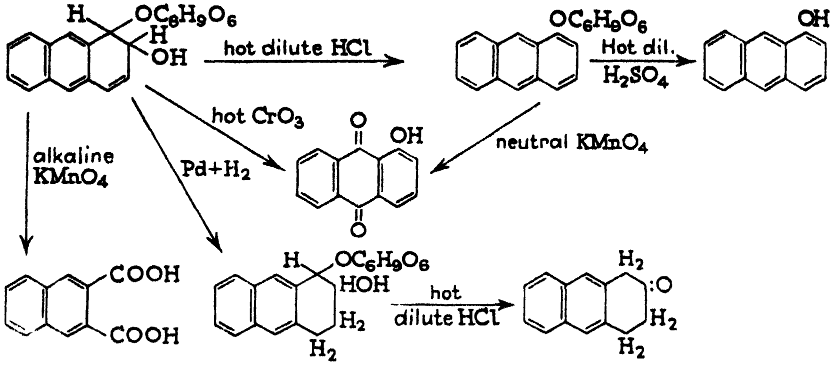
(2) Treatment of the glucuronide with hot dilute HCl gives α-anthrylglucuronide ($[\alpha]_D - 52°$ in dioxan) and this latter on hydrolysis with hot dilute $H_2SO_4$ gives α-anthrol.

(3) α-Anthrylglucuronide gives α-hydroxyanthraquinone on oxidation with neutral $KMnO_4$.

(4) The dihydroxydihydroanthraceneglucuronic acid also gives α-hydroxyanthraquinone on oxidation with hot chromic acid.

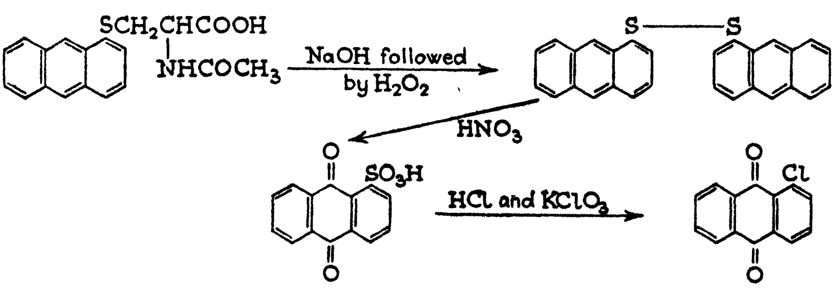
(5) Catalytic reduction of the glucuronide with palladium and hydrogen gives probably 1:2-dihydroxy-1:2:3:4-tetrahydroanthracene glucuronic acid [m.p. 188° ; $[\alpha]_D - 41°$ (Na salt in water)], which is converted to 2-keto-1:2:3:4-tetrahydroanthracene by boiling 2N HCl.

The reactions are summarised below:—



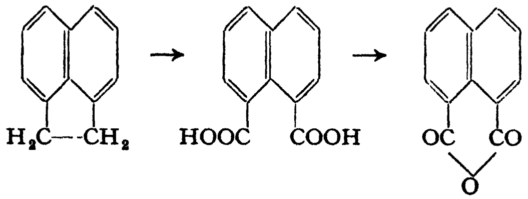
*Anthrylmercapturic Acid.* Rats and rabbits receiving anthracene excrete urine in which there appears to be increased quantities of organic sulphur. The latter was shown by Boyland and Levi (1936) to be due to the presence of anthrylmercapturic acid which they isolated. Unlike the glucuronides, the mercapturic acids of rat and rabbit urine appear to be identical. The acid from rat urine has m.p. 195° and $[\alpha]_D - 6°$ in alcohol and $+ 45°$ in dioxan, whereas that from rabbit urine has m.p. 193° and $[\alpha]_D - 10°$ in alcohol and $+ 46°$ in dioxan.

The mercapturic acid was shown to be α-anthrylmercapturic acid by its conversion to α:α′-dianthryldisulphide, m.p. 191–193°, by alkaline hydrolysis followed by oxidation with hydrogen peroxide. The latter was then converted by $HNO_3$ to anthraquinonesulphonic acid and the sulphonic acid by HCl and $KClO_3$ to α-chloroanthraquinone, a known compound. The reactions are represented as follows:—



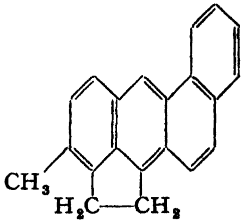
From chloroform extracts of the urine of rats and rabbits receiving anthracene, Boyland and Levi (1936) obtained small amounts of 9:10-anthraquinone. These workers were not prepared to state definitely that the anthraquinone was a true metabolic product of anthracene since it could have been a contaminant of the anthracene ingested.

**Acenaphthene.** Acenaphthene contains a five-membered ring similar to that found in the powerfully carcinogenic compounds cholanthrene and 20-methylcholanthrene



acenaphthene

naphthalic anhydride



20-methylcholanthrene

In rats the five-membered ring undergoes fission and naphthalic anhydride is found in the urine (Chang and Young, 1943). When anthracene, naphthalene and phenanthrene are fed to animals, the urine excreted contains labile compounds which are decomposed by acid to naphthalene, anthracene or phenanthrene as the case may be. This type of labile compound, however, is not found in the urine when acenaphthene is fed, for " acenaphthene urine " does not yield acenaphthene on treatment with acid.

The fact that the five-membered ring of acenaphthene is opened *in vivo*, points to the same possibility in the case of the five-membered ring of cholanthrene and methylcholanthrene.

## CARCINOGENIC HYDROCARBONS

A large number of polycyclic hydrocarbons are known which are capable of inducing cancer in animals. The metabolism of a few of these compounds has been studied and although such studies are fraught with experimental difficulties, some interesting results have been obtained.

The study of the metabolic fate of these compounds is of the greatest importance, since valuable information relevant to the mechanism of their pathological action may be obtained thereby. Knowledge concerning the way in which they may be transformed into harmless compounds is also of great importance, since it has been shown that synthetic hydroxy derivatives of carcinogenic hydrocarbons are either non-carcinogenic or definitely less carcinogenic than their active precursors (see, for example, Fieser and co-workers, 1938; Cook and de Worms, 1937). The majority of carcinogenic hydrocarbons undergo oxidation *in vivo* with the production of phenols (cf. Chalmers and Peacock, 1941) and they therefore fall in line with the simpler aromatic hydrocarbons benzene, naphthalene and anthracene. Only in a very few cases have these phenols been isolated and characterised and their carcinogenic potency tested.

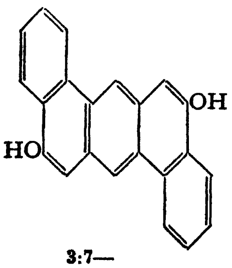
**1:2:5:6-Dibenzanthracene.** The metabolism of 1:2:5:6-dibenzanthracene has been studied by two groups of workers (Boyland and Levi, 1938; Boyland, Levi, Mawson and Roe, 1941; Dobriner, Rhoads and

Lavin, 1939). This compound is a carcinogen of considerable potency and produces tumours in rats, mice, rabbits and fowls (for references to the literature, see Stern and Willheim, 1943).

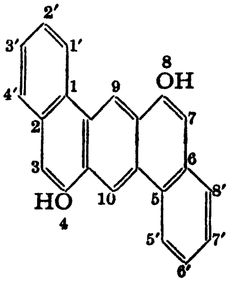
In 1938 Boyland and Levi reported that when dibenzanthracene was fed to rabbits a dihydric phenol, dihydroxy-1:2:5:6-dibenzanthracene could be isolated from the urine. They also showed that there was no positive evidence that " dibenzanthracene urine " contained hydrogenated derivatives carrying asymmetric carbon atoms corresponding to those produced during the metabolism of anthracene. The work of Boyland and Levi (1938) was confirmed by Dobriner *et al.* (1939), who also isolated a dihydric phenol.

The structure of the dihydroxydibenzanthracene excreted by rabbits has not been decided. This is not surprising since 55 isomeric dihydroxydibenzanthracenes are theoretically possible. Cason and Fieser (1940) suggested that the dihydric phenol excreted by rats and mice (Dobriner *et al.*, 1939) might be identical with the 4′:8′-dihydroxy-1:2:5:6-dibenzanthracene which they had synthesised and that the phenol excreted by rabbits might be 3′:7′-dihydroxy-1:2:5:6-dibenzanthracene. The 4′:8′-derivative, however, differs from the compound of Boyland *et al.* (1941). The phenol isolated from rabbit urine by Boyland *et al.* (1941) formed yellow needles, m.p. 340–350° (with decomposition), which gave a violet fluorescence when dissolved in alcohol, acetone or ether and bright green fluorescence in NaOH solution, the latter solution being yellow. It gave no colour with ferric chloride.

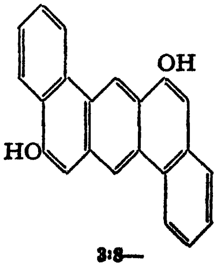
From a number of chemical and spectrographic observations, Boyland *et al.* (1941) came to the conclusion that the phenol was most likely to have one of three formulae, namely, 3:7-, 4:8- or 3:8-dihydroxy-1:2:5:6-dibenzanthracene :—



3:7—



4:8—

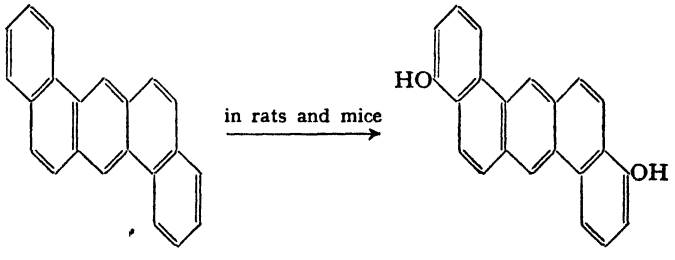


3:8—

That the hydroxyl groups were not in the side rings of the anthracene nucleus was suggested by the fact that permanganate oxidation of the dihydroxy-1:2:5:6-dibenzanthraquinone did not produce anthraquinone-tetracarboxylic acid. Had the hydroxyl groups been in these side-rings, then this acid could be expected. Positions 9 and 10 were also excluded since diacetoxy-, dimethoxy- and dihydroxy-1:2:5:6-dibenzanthraquinone could be prepared. The fact that the dihydroxydibenzanthraquinone does not form a boracetic ether when heated with boracetic anhydride and acetic anhydride, suggests that neither of the OH groups are *peri* (i.e. 4 or 8) to the quinone groups. The evidence appears therefore all in favour of the compound being the 3:7-dihydroxy derivative. However, the above arguments are not conclusive, and Boyland *et al.* (1941) point out that spectrographic evidence points to 4:8-structure.

The dihydroxy compound from rabbits does not produce tumours as does its precursor dibenzanthracene (Boyland *et al.*, 1941 ; Dobriner *et al.*, 1939). The dihydroxydibenzanthracene produced in rabbits is non-carcinogenic for mice, and it appears that the rabbit is endowed with the capacity for converting a carcinogen into a non-carcinogen by oxidation.

The dihydroxydibenzanthracene excreted by rats and mice differs from that excreted by rabbits and is identical with 4′:8′-dihydroxy-1:2:5:6-dibenzanthracene (Dobriner, Rhoads and Lavin, 1942) :—

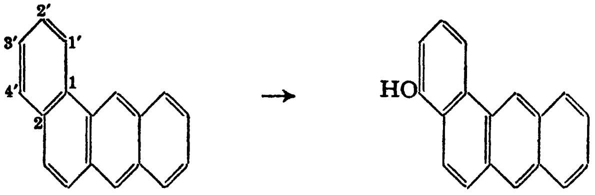


dibenzanthracene 4′:8′-dihydroxydibenzanthracene

It seems unlikely that dihydroxydibenzanthracene is excreted as a glucuronide or an ethereal sulphate for the urine of rats, rabbits and monkeys receiving dibenzanthracene does not yield more dihydroxydibenzanthracene after hydrolysis and autoclaving, than can be obtained by direct ether extraction ; there is also no indication that it forms a mercapturic acid in rats or rabbits (Dobriner *et al.*, 1942).

Regarding its mode of action, it is suggested that dibenzanthracene interferes with that function of the liver which is concerned with the metabolism of vitamin A (Abels, Gorham, Eberlin, Halter and Rhoads, 1942). A competition is envisaged between vitamin A and dibenzanthracene for some substance, possibly a protein, to which vitamin A may be bound in the liver. Dibenzanthracene does not impair the hepatic storage of riboflavin and the utilisation of coenzyme I as does the carcinogen butter yellow (see p. 152). From this it appears possible that different carcinogens selectively inhibit different reactions which occur in liver cells (Abels *et al.*, 1942).

**1:2-Benzanthracene** is oxidised in rats and mice for Berenblum and Schoental (1943*b*) have isolated and characterised the oxidation product as 4'-hydroxy-1:2-benzanthracene. In this case the oxidation has definitely been proved to take place in the side-ring of the anthracene nucleus.

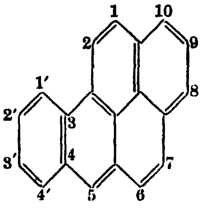


1:2-benzanthracene

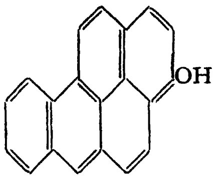
4'-hydroxy-1:2-benzanthracene

**3:4-Benzpyrene.** It is well known that coal tar is capable of producing cancers, and from it there has been isolated a pure compound of extremely great carcinogenic potency. This compound was identified by Cook, Hewitt and Hieger (1933) as 3:4-benzpyrene. It has not been claimed, however, that benzpyrene is the sole active agent in coal tar and others may be present. Derivatives of dibenzanthracene have not been found in the carcinogenically active fractions of coal tar and hence the carcinogenicity of coal tar cannot be partly due to compounds of the dibenzanthracene type. 3:4-Benzpyrene shows greatest carcinogenic activity in mice. Rabbits seem to be less susceptible to the stimulus of this carcinogen.

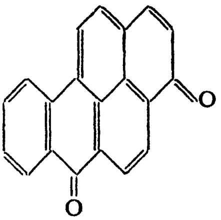
When benzpyrene is administered to rats and mice, the faeces contain a substance showing a blue-green fluorescence (Chalmers, 1938), whereas the bile shows a blue fluorescence (Peacock, 1936, 1940). Benzpyrene itself in organic solvents shows a violet fluorescence. The metabolic product appearing in the bile was designated " BPX ", whilst that in the faeces was indicated by " BPF ". Chalmers and Kirby (1940) found approximately 1% of injected benzpyrene to be eliminated unchanged by the rat in the faeces. On purification, " BPX " and " BPF " appeared to be identical (Chalmers and Crowfoot, 1941). The latter workers isolated a small amount of " BPX " in a crystalline state. It was phenolic and had m.p. 190–196° (decomp.). It was shown to be different from both 4'-hydroxy-3:4-benzpyrene (m.p. 217°) and 6-hydroxy-3:4-benzpyrene (m.p. 195–196°). Crystallographic data pointed to a number of possibilities. The most likely positions for the hydroxyl group were 7, 8, 9, 1', 2' or 3'; 1 and 2 were unlikely, 6 and 4' were eliminated by direct comparison, whilst 5 and 10 were just possible. The compound was finally identified by Berenblum, Crowfoot, Holiday and Schoental (1943) as 8-hydroxy-3:4-benzpyrene, which was prepared synthetically by Berenblum and Schoental (1943*a*). Berenblum *et al.* (1943) also isolated from the faeces of rats receiving 3:4-benzpyrene a quinone which was identified as 3:4-benzpyrene-5:8-quinone.



3:4-benzpyrene



8-hydroxy-3:4-benzpyrene



3:4-benzpyrene-5:8-quinone

Using the technique of fluorescence chromatography of tissue extracts, Weigert and Mottram (1943) have shown that benzpyrene is not directly metabolised by mice to 8-hydroxybenzpyrene, and they are of the opinion that at least three intermediate stages (*in vivo* and *in vitro*) are involved. They consider " BPX " and " BPF " to be different metabolites and both are different from 8-hydroxybenzpyrene (cf. Chalmers and Crowfoot, 1941). They also distinguish two further products which they designate (BPX) and (BPF). (BPX) and (BPF) are formed from " BPX " and " BPF " respectively during adsorption of the extracts on alumina. The 8-hydroxy-3:4-benzpyrene of Berenblum *et al.* was obtained from faecal extracts by chromatography followed by sublimation *in vacuo*, and therefore the probability exists that it is not a direct metabolic product of benzpyrene in the animal; it may be derived from a precursor during the chromatographic and sublimation procedures. The immediate precursor of 8-hydroxybenzpyrene according to Weigert and Mottram is probably (BPF). The latter is produced from " BPF " during chromatography on alumina. " BPF " is produced from " BPX " *in vivo*, since the conversion of " BPX " to " BPF " was demonstrated by Weigert and Mottram in the large intestine of the living mouse after passage through the ileo-caecal valve. " BPF " is also produced from " BPX " during autolysis of tissues from benzpyrene-treated mice. The transformations[1] leading to 8-hydroxybenzpyrene

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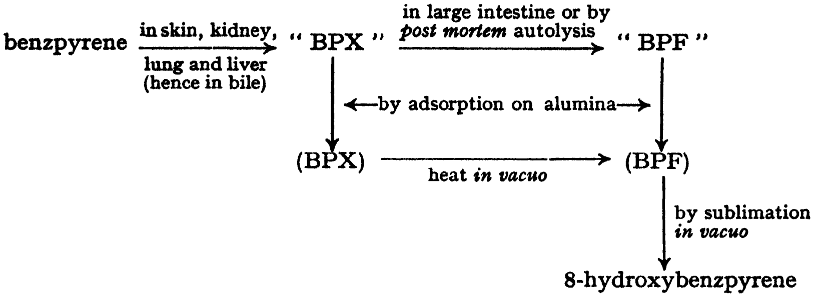
[1] In a more recent paper, Weigert (1945) has produced evidence as to the chemical nature of the intermediary metabolites of benzpyrene. In this paper, he considers that 8-hydroxybenzpyrene is formed as follows:—

$$\begin{tikzcd} BP \arrow[r] & X_1 \arrow[r] & F_1 \arrow[r] & F_2 \arrow[r] & 8\text{-HO-BP} \\ & X_2 \end{tikzcd}$$

$X_1$, formed in the liver, enters the bile, a small amount being transformed into $X_2$, but the main bulk of $X_1$ is changed into $F_1$ after its passage through the ileo-caecal valve. In the later stages of the metabolism, more and more of $F_2$ appears in the faeces. $F_1$ and $F_2$ are phenolic, whereas $X_1$ and $X_2$ are not phenolic. $F_2$ has the same absorption spectrum as 8-hydroxybenzpyrene and may be identical with this phenol or a loose conjugate of it. $F_2$ is closely similar to $F_1$. It is suggested that the non-phenolic $X_1$ is 8:9-dihydroxy-8:9-dihydro-3:4-benzpyrene (cf. anthracene, p. 48), or one of its conjugates.

according to Weigert and Mottram are given in the following scheme :—

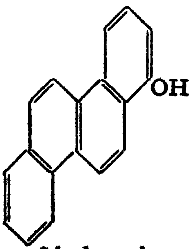


From the above scheme it is clear why " BPX " is found in the bile and " BPF " in the faeces. The transformation of " BPX " to " BPF " does not take place until the ileo-caecal valve has been passed, and hence " BPX " could be expected to be excreted in the bile. Since only the conversion of benzpyrene to " BPX " takes place in the cells of the body, then this is the only stage concerned in the problem of benzpyrene carcinogenesis (Weigert and Mottram). It is worthy of note that benzpyrene is eliminated in rats and mice to a considerable extent in the faeces (see p. 48).

### OTHER HYDROCARBONS

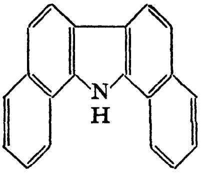
Chalmers and Peacock (1941) have shown that a number of other carcinogenic hydrocarbons undergo change in the organism. They found that in the fowl injected **cholanthrene** and **methylcholanthrene** underwent changes to fluorescent derivatives analogous to the " BPX " of benzpyrene. These derivatives were present in the bile. Amongst non-carcinogenic hydrocarbons, **pyrene**, **2′:6-dimethyl-1:2-benzanthracene**, **2′:7-dimethyl-1:2-benzanthracene** and **fluoranthene** also gave rise to fluorescent derivatives on injection into the fowl which were excreted in the bile. Unlike benzpyrene, **3:4-benzphenanthrene** and all the above-mentioned hydrocarbons were not excreted unchanged in recognisable amounts by the fowl but underwent transformation. Benzphenanthrene, however, did not give rise to fluorescent derivatives in the bile.

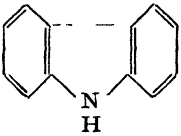
The non-carcinogenic hydrocarbon **chrysene** is converted in the rat into 3-hydroxychrysene which is found in the faeces (Berenblum and Schoental, 1945). It does not increase the ethereal sulphate output in rats (Elson *et al.*, 1945).



**3-hydroxychrysene**

**3:4:5:6-Dibenzcarbazole** has been studied by Boyland *et al.* (1941). They found this carcinogen to cause an increased output of ethereal sulphates in rabbits, indicating that the dibenzcarbazole is excreted as a sulphuric ester, probably of a hydroxydibenzcarbazole with a characteristic fluorescence. **Carbazole** itself is oxidised in the dog to a hydroxycarbazole of unknown constitution and the output of ethereal sulphates is increased (Klingenberg, 1891).

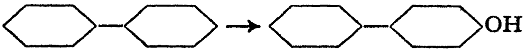

3:4:5:6-dibenzcarbazole


carbazole

## DIPHENYL AND OTHER BENZENOID COMPOUNDS

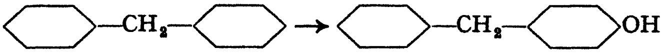
The fate of compounds of this type is of interest since some are related to the synthetic oestrogens derived from stilbene, e.g. 4:4′-dihydroxy-*s*-diphenylethylene (stilboestrol). However, little information is available regarding the metabolism of this type of hydrocarbon, except to show that bio-oxidation of the ring with formation of phenols does take place, and they are therefore similar to benzene in this respect.

**Diphenyl.** When diphenyl is injected into rabbits it is converted in part to 4-hydroxydiphenyl (Stroud, 1940).

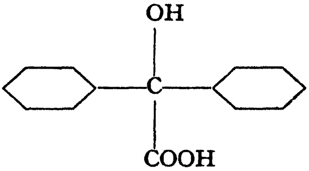


About 25% of the injected diphenyl can be recovered from the urine as the phenol which is mainly excreted free; only a small proportion (one-fifth) of the phenol is excreted combined and none of the hydrocarbon is excreted unchanged. A similar change occurs in the dog which excretes the hydroxydiphenyl as an ethereal sulphate (Klingenberg, 1891). Neither diphenyl nor its metabolic product has any oestrogenic activity. Rats receiving a diet containing 1% of diphenyl, also excrete a metabolite containing sulphur (West, 1940), which suggests that diphenyl may be excreted as a mercapturic acid. Unless extra sulphur in the form of *l*-cystine or *dl*-methionine, but not taurine or $Na_2SO_4$, is added to the diet, rats receiving 1% diphenyl in their diet do not grow normally (cf. bromobenzene and naphthalene).

**Diphenylmethane.** Stroud (1940) has shown that this hydrocarbon when injected into rabbits is converted into 4-hydroxydiphenylmethane which is excreted in the urine both free and combined. 14·7% of the injected hydrocarbon can be accounted for as the phenol, 13·1% being free and 1·6% combined. The nature of the combined fraction has not been elucidated. Neither diphenylmethane nor its phenolic metabolite has any oestrogenic activity. Hydroxydiphenylmethane is also formed in the dog, but this animal does not oxidise *p*-bromodiphenylmethane (Klingenberg, 1891).

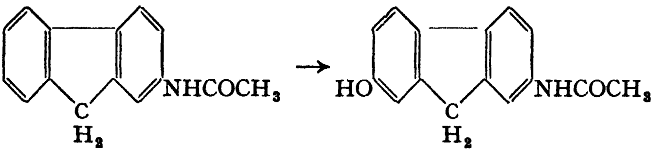


**Benzilic acid**, which is closely related to diphenylmethane, is excreted unchanged by rabbits (Sieberg and Harloff, 1919).



benzilic acid

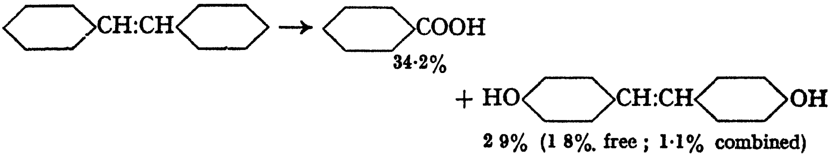
**Diphenylenemethane** or **fluorene** does not appear to have been studied but a derivative, **2-acetaminofluorene,** is oxidised in rats to 2-acetamino-7-hydroxyfluorene (Bielschowsky, 1944) :—



2-Acetaminofluorene is of interest in that when fed to rats it induces distant tumours in the liver and mammary glands. The compound damages the liver and therefore excess endogenous oestrogens are not destroyed. The excess of endogenous oestrogens is apparently the proximate cause of the breast tumours in animals fed with this compound (Bielschowsky, 1944*a*).

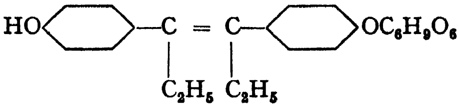
**Triphenylmethane,** $(C_6H_5)_3CH$. In the opinion of Miriam, Wolf and Sherwin (1927) triphenylmethane is largely excreted unchanged in dogs and rabbits. These workers recovered 27·5% and 35% of unchanged triphenylmethane from the urine of rabbits and dogs respectively after the oral administration of the hydrocarbon. The fate of the unrecovered fraction is apparently obscure.

**Stilbene.** A number of hydroxy derivatives related to stilbene have been found to possess oestrogenic activity, and most of these artificial oestrogens are derivatives of 4:4′-dihydroxystilbene and 4:4′-dihydroxy-*s*-diphenylethane. The biological conversion of stilbene derivatives into hydroxy derivatives is therefore of considerable interest. On injection into rabbits the main product of stilbene metabolism appears to be benzoic acid, indicating that the molecule splits *in vivo* at the double bond. A small amount of 4:4′-dihydroxystilbene, however, is formed and excreted both free and combined (Stroud, 1940, 1939). No unchanged stilbene is excreted. About 37% of the injected stilbene can be accounted for by these metabolites.



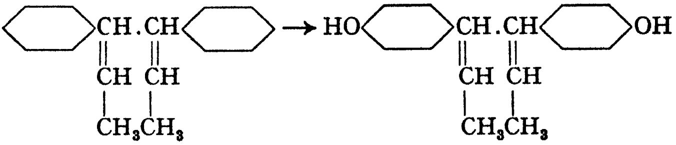
Other possible metabolites of stilbene are *p*-hydroxybenzoic acid and monohydroxystilbene, but their occurrence has not been investigated.

The artificial oestrogen, **stilboestrol**, on injection into rabbits is partly converted into a monoglucuronide (m.p. 175° : $\left[\alpha\right]_D^{20}$ — 56·6° in alcohol) which yields stilboestrol on acid hydrolysis. Mazur and Shorr (1942) were able to isolate from the urine of rabbits as much as 30% of the administered stilboestrol as this glucuronide.

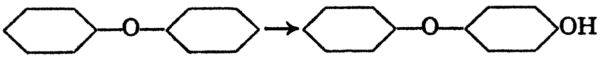


stilboestrol monoglucuronide

**γ:δ-Diphenylhexa-2:4-diene.** Stroud (1940) is of the opinion that this compound, like stilbene, forms a small amount of dihydroxy derivative.



Despite lack of information regarding the metabolism of diphenyl and stilbene derivatives, it is possible from the above paragraphs to make one or two tentative generalisations, at the same time bearing in mind that a complete balance sheet for the fate of these compounds has not yet been made. Firstly, when the number of carbon atoms separating the two benzene rings is 0 or 1, then oxidation in the *para* position takes place only in one ring. This also applies when the atom separating the phenyl groups is oxygen, since Stroud (1940) has shown that diphenylether on injection into rabbits results in the excretion of free and combined 4-hydroxydiphenylether.



Secondly, when the number of carbon atoms separating the phenyl groups is 2, as in stilbene or γ:δ-diphenylhexa-2:4-diene, then oxidation takes place in the *para* position in both rings. No studies have been made so far on compounds where 3 or more carbon atoms separate the rings.

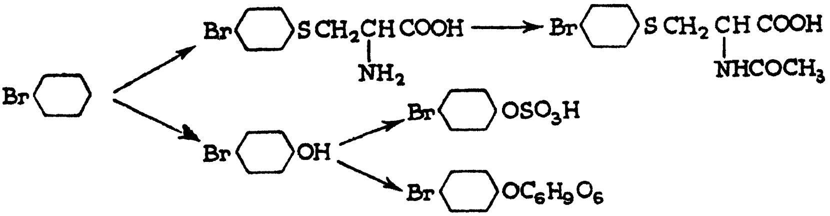
## *Chapter Four*

# The Metabolism of Halogenated Aromatic Hydrocarbons

INVESTIGATION of the fate of halogenated hydrocarbons has centred mainly round the formation of mercapturic acids and the consequent relationship to cysteine and sulphur metabolism. The production of mercapturic acids, however, is only one aspect of the metabolism of these hydrocarbons and other aspects such as their oxidation to phenols have been somewhat neglected.

The first records of the study of the fate of halogenated hydrocarbons in the animal body are to be found in the work of Baumann and Preusse (1879) and of Jaffe (1879). The more recent work of Hele and of Stekol and their co-workers has done much to elucidate the problem of mercapturic acid synthesis.

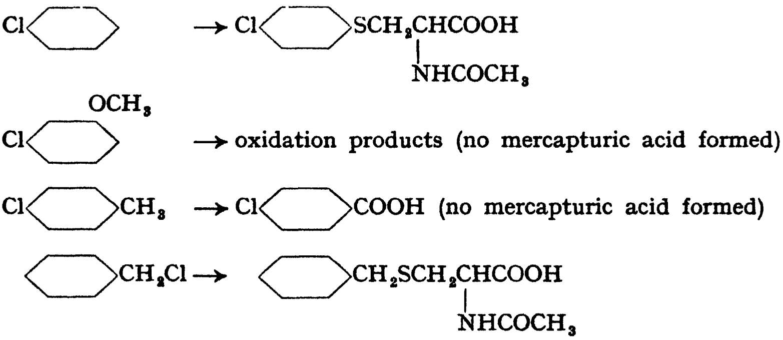
When halogenated hydrocarbons are fed to animals they undergo two types of reactions, namely, oxidation to a phenol which is excreted combined with both glucuronic and sulphuric acids, and synthesis with the formation of a mercapturic acid. The general reactions may be exemplified by the case of bromobenzene.



According to Baumann and Preusse (1881) the oxidation process may proceed further and halogenated dihydric phenols may also be excreted.

The types of halogenated hydrocarbon which may undergo the mercapturic acid synthesis are important. It has already been mentioned in an earlier chapter that benzene, polycyclic hydrocarbons and possibly diphenyl may undergo this synthesis. The monohalogen benzenes, **fluorobenzene, chlorobenzene, bromobenzene** and **iodobenzene** all form mercapturic acids (Baumann, 1883; Young and Zbarsky, 1944), and this is probably true for *o*- and *m*-**dichlorobenzene** (Callow and Hele, 1926). The position of *p*-**dichlorobenzene** is not clear; Baumann (1883) states that this compound does not form a mercapturic acid and this is likely to be true since the acetylcysteine group normally conjugates in the *para* position to the halogen and this position is occupied in *p*-dichlorobenzene. There is evidence that the monohalogen naphthalenes form mercapturic acids (Baumann) and this is probably true for the halogenated polycyclic hydrocarbons.

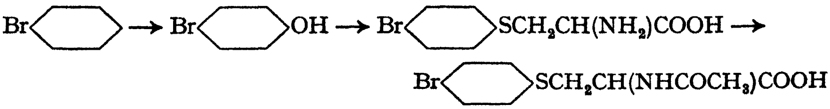
Whereas the monohalogenated benzenes are mercapturic acid formers *in vivo*, the nuclear substituted halogenated toluenes are not. Baumann (1883) states that the mono- and di-chlorotoluenes do not form mercapturic acids and Callow and Hele (1926) show that **o-chlorotoluene** has no influence on sulphur metabolism in the dog. On the other hand, if the halogen occurs in the side-chain, as in **benzyl chloride**, mercapturic acid formation takes place with the elimination of the halogen (Stekol, 1939). If the halogenated benzene contains other groups attached to the ring, then its metabolism takes a totally different path and mercapturic acids are not formed, for Coombs and Hele (1926) showed that the introduction of methoxyl, acetamino or hydroxyl groups into the chloro- or bromobenzene molecules abolishes the ability to form mercapturic acids. The following compounds do not give rise to mercapturic acids in the dog :—*o*-, *m*- and *p*-chlorophenol, *o*- and *p*-bromoanisole, *m*-chloroanisole, *o*- and *p*-chloroacetanilide.


Cl
Cl
SCH₂CHCOOH
NHCOCH₃
OCH₃
Cl
oxidation products (no mercapturic acid formed)
Cl
CH₃
Cl
COOH (no mercapturic acid formed)
CH₂Cl
CH₂SCH₂CHCOOH
NHCOCH₃

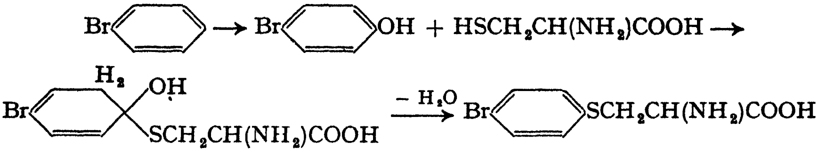
Dogs, rabbits, cats, rats and mice (see various papers by Stekol; Conway, 1937) are capable of carrying out the mercapturic acid synthesis, but the pig is unable to do so. The pig, in fact, does not readily use compounds containing sulphur for conjugation purposes and can only produce ethereal sulphates to a small extent (Coombs and Hele, 1927).

The formation of mercapturic acids in the animal body raises a number of important questions. In the first place, since the ethereal sulphates of phenols are frequently excreted with mercapturic acids and since cysteine is oxidised *in vivo* to inorganic sulphate, what relation do the ethereal sulphates bear to the mercapturic acids? Secondly, since the cysteine of the mercapturic acids occurs acetylated, does conjugation of the hydrocarbon occur with preformed acetylcysteine or with cysteine, the acetylation of the conjugated molecule being a secondary reaction? Thirdly, is the cysteine used in mercapturic acid synthesis of endogenous or exogenous origin and what other sulphur-containing amino acids if any can replace it?

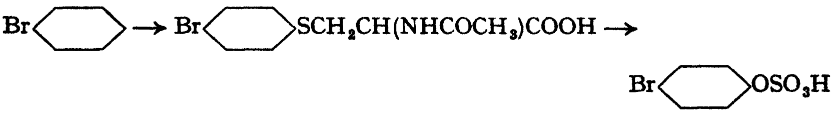
*The Linking of Sulphur to Nuclear Carbon* in vivo. Regarding the first question, Sherwin (1922) expresses the opinion that the first product of bromobenzene metabolism is bromophenol, which then combines with cysteine to form *p*-bromophenylcysteine. The latter is then acetylated :—



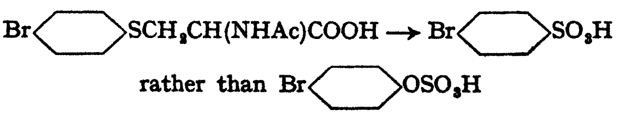
From this scheme it appears that the ethereal sulphate, the glucuronide and the mercapturic acid are derived from bromophenol. A similar scheme was suggested by Rhode (1923), who visualised the aromatic ring being partly reduced in the process. Rhode's scheme is as follows :—



The reduction of the aromatic ring is not unreasonable since this process has been proved to take place with anthracene and probably with naphthalene and phenanthrene (see p. 48). Furthermore, Rhode showed that bromobenzene fed to rabbits resulted in the excretion of more ethereal sulphate than did an equivalent dose of *p*-bromophenol. His figures show that 35–45% of bromobenzene (dose equivalent to 0·2 g. phenol/kg.) formed an ethereal sulphate, whereas only 25–35% of an equivalent dose of *p*-bromophenol was excreted as an ethereal sulphate. However, this type of theory is likely to be untenable since administration of *p*-bromophenol does not lead to the excretion of *p*-bromophenylmercapturic acid, and it is probable that the pathways of metabolism of bromophenol and of bromobenzene are totally different (Coombs and Hele, 1926). On the other hand, it is possible that the first product formed is the mercapturic acid which is then oxidised to an ethereal sulphate thus :—



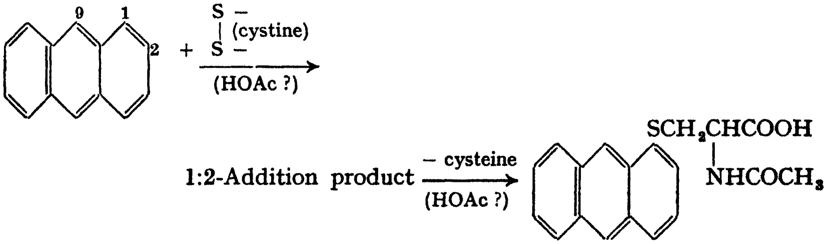
On theoretical grounds the transformation of the mercapturic acid into an ethereal sulphate would be an extremely difficult reaction to carry out because it involves the breaking of the strong link between sulphur and nuclear carbon and replacing it by a carbon-oxygen link, when normally one would expect the formation of a sulphonic acid thus :—



The *in vivo* oxidation of the mercapturic acid to an ethereal sulphate or to a phenol, probably does not take place since Callow and Hele (1927) have shown that the administration of *p*-chloro- or *p*-bromophenylmercapturic acid to dogs does not result in an increased output of ethereal sulphate. Similar results were obtained by Lawrie (1931) in rats and rabbits given *p*-iodophenylmercapturic acid.

From the evidence available the most probable pathway of the metabolism of bromobenzene is its simultaneous conjugation and oxidation, the primary products being *p*-bromophenylcysteine and *p*-bromophenol. The secondary reactions are probably acetylation of bromophenylcysteine and conjugation of bromophenol with sulphuric and glucuronic acids.

A possible mechanism for the combination of the aromatic ring with cysteine *in vivo* has been suggested by the work of Cason and Fieser (1940). They were considering the case of anthracene which forms 1-anthrylmercapturic acid in the body and point out that the fixation of sulphur by means of chemical substitution (using sulphur monochloride) takes place at the 9-position and therefore the formation of 1-anthrylmercapturic acid in the body is likely to be an addition reaction rather than a substitution. Cason and Fieser suggest that, *in vivo*, anthracene first combines with cystine to form a 1:2-addition product which then loses cysteine, leaving the mercapturic acid. They did not formulate the addition product and their scheme is here reproduced without modification :—



In the case of benzyl chloride, the intermediate stage between it and S-benzylcysteine is not clear ; possible intermediates such as benzyl alcohol and benzaldehyde do not themselves form mercapturic acids but are oxidised to benzoic acid and excreted as hippuric acid (Stekol 1939). Any mechanism explaining the synthesis of mercapturic acids in which the sulphur atom is attached to a nuclear carbon atom must also explain the synthesis of benzylmercapturic acid. The difficulties are obvious, for the only mechanism which will explain both is one of substitution.

*The Acetylation of Cysteine Derivatives* in vivo. When *p*-bromophenyl-*l*-cysteine, phenyl-*l*-cysteine and S-benzyl-*l*-cysteine are fed to animals they are acetylated and are converted to *p*-bromophenylmercapturic acid, phenylmercapturic acid and N-acetyl-S-benzyl-*l*-cysteine, respectively (Stekol, 1937–8*a* ; 1937 ; Zbarsky and Young, 1943*c*). These results can be taken as supporting the view that acetylation of phenylcysteine derivatives is a secondary reaction in mercapturic acid synthesis. According to Stekol (1937–8 and 1938) there are two possible ways in which acetylated cysteine derivatives may be formed *in vivo.*

In the first place, the cysteine compound may be directly acetylated, or, secondly, the mercapturic acid may be the product of the interaction of ammonia and pyruvic acid with a derivative of $\alpha$-keto-$\beta$-thiopropionic acid according to the mechanism suggested by Knoop and Blanco (1925) for the synthesis of amino acids *in vivo*.[1] The results of du Vigneaud, Wood and Irish (1939) on the metabolism of the *d*- and *l*-isomers of S-benzylcysteine, whilst indicating that one enantiomorph is convertible to the other *in vivo*, possibly through the mechanism of Knoop and Blanco,[1] also demonstrate the direct acetylation of S-benzyl-*d*-cysteine in the body. It seems probable, therefore, that direct acetylation of arylcysteine derivatives does take place *in vivo* (see Stekol, 1941*b*).

*The Source of Cysteine for Mercapturic Acid Synthesis.* Although the preformed cysteine of the diet cannot be used directly for the synthesis of mercapturic acids the growing organism, at least, cannot continue to flourish and synthesise mercapturic acid unless cysteine or certain other sulphur-containing amino acids are present in the diet in adequate amounts. Dietary sulphur is not the immediate source of sulphur utilised in the detoxication of bromobenzene, since the extent of the synthesis of bromophenylmercapturic acid in well-fed dogs is independent of the time of ingesting food (Stekol and Foy, 1937). Cysteine for mercapturic acid synthesis is derived from endogenous sources (Conway, 1937), and the sulphur-containing amino acids of the diet affect the synthesis only in so far as they determine the nutritional state of the animal. Young fasting animals can synthesise mercapturic acids from dietary bromobenzene or naphthalene at the expense of tissue cysteine, but their growth is eventually checked unless a source of cysteine is found sufficient to cover the needs of growth and detoxication (Stekol, 1937–8*b*). In adult animals on diets low in sulphur, the administration of cysteine, *l*-cystine or *dl*-methionine increases mercapturic acid synthesis (Stekol, 1937*a*) ; glutathione and taurine are ineffective (Stekol, 1938) ; caseinogen is not an efficient source of either cysteine or methionine for the detoxication of bromobenzene (Stekol, 1943).

*Quantitative Aspects.* About one-third to one-half of the chloro-, bromo-, or iodobenzene fed to the dog is excreted as a mercapturic acid and the remainder is presumably oxidised to phenols. In the case of the dog, of a dose of 4 g. iodobenzene, some 47% is excreted as a

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[1] Knoop's acetyl synthesis of amino acids suggests that an amino acid may be formed *in vivo* from a keto acid by the interaction of the latter with pyruvic acid and ammonia, there taking place an internal oxidation and reduction followed by loss of $CO_2$, the product being the N-acetyl derivative of the amino acid

$$R.COCOOH + NH_3 + CH_3COCOOH \longrightarrow R.CH(NHCOCH_3)COOH + CO_2 + H_2O$$

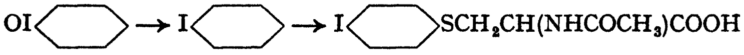
The inversion of S-benzyl-*d*(−)cysteine to S-benzyl-*l*(+)acetyl-cysteine would be something as follows :—

$$d(-)C_6H_5CH_2SCH_2CH(NH_2)COOH \xrightarrow{\text{oxidative deamination}} C_6H_5CH_2SCH_2COCOOH$$

$$\xrightarrow[\text{by Knoop's acetyl synthesis}]{NH_3 + CH_3COCOOH} l(+)C_6H_5SCH_2CH(NHCOCH_3)COOH$$

mercapturic acid (as indicated by the neutral sulphur output of the urine), and some 25–30% is excreted as ethereal sulphates (Hele, 1924; Coombs and Hele, 1926). In the pig, iodobenzene forms no mercapturic acid and only 18–19% can be accounted for as ethereal sulphate; the rest is probably oxidised and excreted as a glucuronide as indicated by the reducing power of the urine (Coombs and Hele, 1927). From the values of the neutral sulphur and ethereal sulphate outputs of dogs receiving chlorobenzene or bromobenzene, Coombs and Hele showed that some 47% of the former was excreted as mercapturic acid and about 28% as sulphates, whereas for the latter the figures were 35–36% and 19–25% respectively. Figures for yields of mercapturic acids from dogs have been given by Baumann (1883), who obtained 26–27 g. of the conjugate for every 100 g. of chlorobenzene fed, 26–28 g. for bromobenzene and 18 g. for iodobenzene. In the case of fluorobenzene, the yields are much lower, for Young and Zbarsky (1944) were only able to isolate from the urine of rats 1–2% of injected fluorobenzene as crystalline *l*-fluorophenylmercapturic acid.

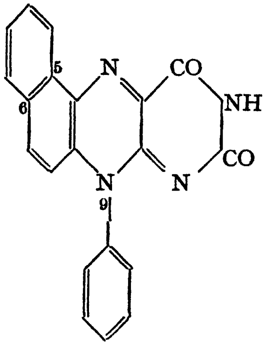
**Iodosobenzene.** According to Luzzato and Satta (1909) iodosobenzene yields *p*-iodophenylmercapturic acid when fed to dogs or rabbits; the urine also contains much ionised iodine. Iodosobenzene is apparently first reduced *in vivo* to iodobenzene, which is then partly excreted as the mercapturic acid.



### The Mercapturic Acid Synthesis and Keratin-containing Tissues

It is a well-recognised fact that tissues such as skin, hair, wool, nail, etc., are rich in cystine. It is therefore reasonable to suppose that the administration to animals of compounds which form mercapturic acids may result in the appearance of such conjugates in the hair, skin or nails. Recent work has suggested that this may actually be the case. It has been known for a considerable time that the administration of arsenic compounds is followed by the appearance of arsenic in the hair, skin and nails. Arsenious oxide and 3-amino-4-hydroxyarsenoxide form compounds with cysteine (Johnson and Voegtlin, 1930) and cysteine delays the toxic effects of arsenious oxide (Labes, 1929). The combination of arsenic with cysteine may therefore explain why arsenic appears in skin, hair and nail. It has also been suggested that bromobenzene when applied to the skin of the mouse is converted to the mercapturic acid in the skin and the conjugate is then excreted in the urine (Crabtree, 1944).

A striking example of the possible relationship between the mercapturic acid synthesis and the elaboration of hair keratin from cystine is afforded by the fate of 9-phenyl-5:6-benzo*iso*alloxazine in the rat (Haddow, Elson, Roe, Rudall and Timmis, 1945). Injection of this compound into albino rats produces an orange-yellow pigmentation of the hair which contains either the original compound or a compound spectroscopically similar to it. It also appears probable that phenylbenzo*iso*alloxazine is excreted as a mercapturic acid and it may be that phenylbenzo*iso*alloxazine is transported to the hair by combining with the cysteine which is involved in the elaboration of keratin. The 9-methyl-, 9-ethyl-, 9-*n*-propyl-, 9-*n*-butyl- and 9-*n*-amyl-5:6-benzo*iso*alloxazines also produce pigmentation of the hair of the rat, but very much less than in the case of the phenyl derivative.



It should be noted that the hair and skin are possible channels of excretion of mercapturic acids.

## Selenium Compounds

The possibility that selenium may take the place of sulphur in the synthesis of mercapturic acids is indicated by the observations of Moxon, Schaeffer, Lardy, DuBois and Olson (1940). Steers feeding on a seleniferous pasture absorb selenium and their blood has a comparatively high selenium content. They eventually suffer from selenium poisoning. The feeding of bromobenzene, however, reduces the selenium content of the blood and there is a rise in urinary selenium which may be present as a selenium mercapturic acid.

Westfall and Smith (1941), in experiments on rabbits fed on seleniferous wheat, have found that the selenium excreted in the urine is mainly in organic combination, appearing in the ethereal sulphate and neutral sulphur fractions. They found, however, that the feeding of bromobenzene to selenised rabbits did not increase the urinary selenium output nor did it cause a decrease in tissue selenium, and they conclude that bromobenzene is not an effective agent for removing selenium from the body and that there is no substantial basis for its use in therapy. These workers also prepared *p*-bromophenylmercapturic acid from the urine of selenised rabbits fed bromobenzene and found only small amounts of selenium in the crude acid. They conclude, therefore, that little selenium is likely to be eliminated as the selenium analogue of *p*-bromophenylmercapturic acid. Although selenium is known to occur in the proteins of seleniferous grains (see White, 1941), there is as yet no definite proof that it can form in the body compounds analogous with the mercapturic acids.

TABLE 7

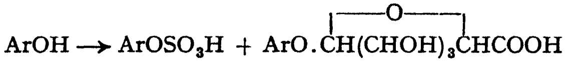
*Biosynthetic Mercapturic Acids*

| Compound | M.P. | $[\alpha]_D$ in alcohol | Reference |
|---|---|---|---|
| *l*-phenylmercapturic acid | 142° | − 22° | Zbarsky and Young (1943*a* and *b*) |
| *l*-*p*-fluorophenylmercapturic acid | 158–9° | − 19° | Young and Zbarsky (1944) |
| *l*-*p*-chlorophenylmercapturic acid | 153–4° | laevo-rotatory | Jaffe (1879) |
| *l*-*p*-bromophenylmercapturic acid | 152–3° | − 6·7° (+ 6·4° in dil. NaOH) | Baumann and Preusse (1881); Baumann (1882) |
| *l*-*p*-iodophenylmercapturic acid | 152–3° | − 10·7° | Baumann and Schmitz (1895) |
| *l*-benzylmercapturic acid | 147–8° | − 43·5° | Stekol (1938); du Vigneaud, Wood and Irish (1939) |
| *dl*-benzylmercapturic acid | 154–5° | — | du Vigneaud *et al.* (1939). |
| N-acetyl-S-benzyl-*l*-*homo*-cysteine | 132° | + 6° | du Vigneaud *et al.* (1939). |
| *l*-α-naphthylmercapturic acid | 170–1° | − 25° | Bourne and Young (1934) |
| *l*-α-anthrylmercapturic acid | 195° | − 6° | Boyland and Levi (1936) |

## *Chapter Five*

# The Metabolism of Phenols

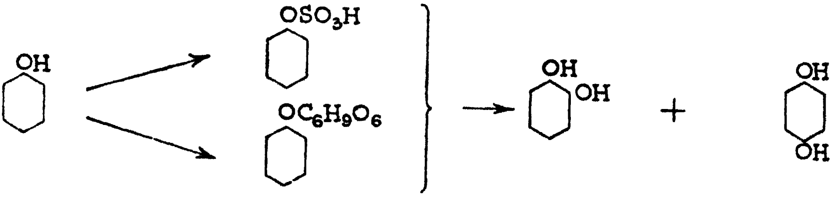
THE fate of phenols in the animal body is a relatively straightforward matter for, in general, they are converted into arylsulphuric acids and arylglucuronides thus :—



$$\mathrm{ArOH} \rightarrow \mathrm{ArOSO_3H} + \mathrm{ArO.}\overbrace{\mathrm{CH(CHOH)_3C}}^{\mathrm{O}}\mathrm{HCOOH}$$

A proportion of the phenol is often excreted in the free state and in the case of monohydric phenols, a small part may be further oxidised to polyhydric phenols. The arylsulphuric acid or ethereal sulphate mechanism appears to be specific for phenolic substances (including heterocyclic phenols, hydroxy purines and hydroxy pyrimidines), and no other hydroxyl group is known which will combine with sulphuric acid *in vivo*. It is well known that compounds of the natural carbohydrates occur which contain sulphate groups, but no carbohydrate is known which when fed to an animal is excreted combined with sulphuric acid. Ethereal sulphate groups are found in natural polysaccharides such as heparin, chondroitin sulphate, an acid polysaccharide from gastric mucin, the galactose-containing polysaccharides from carrageen moss (Buchanan, Percival and Percival, 1943) and agar (Jones and Peat, 1942).

**Phenol.** Since phenol is a compound of considerable importance in all fields of medical science, its fate in the animal body has been the subject of numerous investigations. When it is administered to an animal[1] it is partly oxidised, partly conjugated with sulphuric and glucuronic acids and partly excreted unchanged. Products which may be found in the urine are unchanged phenol, phenylsulphuric acid, phenylglucuronide, catechol and quinol. The latter two compounds may also be conjugated. The metabolism of phenol may therefore be represented as follows :—



Catechol and quinol are only detectable when large doses of phenol are fed (Baumann and Preusse, 1879*a*). The amount of phenol oxidised and the relative amounts of phenylsulphuric and phenylglucuronic acids formed also depend on the dose level. In studies on the excretion of phenol there is always a certain proportion of the dose fed which cannot be accounted for. This fraction is usually said to be oxidised, although how this oxidation takes place and what its products are (other than minute amounts of catechol and quinol) remain obscure. The early work of Tauber (1878) showed that the amount of phenol " oxidised " in dogs depends upon the dose. Small doses cannot be recovered at all from the urine, whereas with relatively large doses, 50% or more can be accounted for by metabolites in the urine. According to Dubin (1916) some 65% of ingested phenol is eliminated by dogs, and 30–70% of that eliminated is in a combined form.

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[1] During the first 15 minutes after the introduction of phenol into the intestine of the rat, it is absorbed at the rate of 14 mg./100 g. body weight/hour (Mareque and Marenzi, 1938).

Working on rabbits, Williams (1938) showed that when small doses of phenol are fed the ethereal sulphate conjugation accounts for the greater part of the combined phenol eliminated, and that at a dose level of 25 mg./kg. more than 40% of the ingested phenol is excreted combined with sulphuric acid. As the dose increases, the percentage combined with sulphate decreases rapidly, reaching 20% at the 100 mg./kg. level, and remains fairly constant at this figure right up to the toxic dose, which is about 500 mg./kg. As the dose is increased, the glucuronic acid conjugation becomes more pronounced. It appears that there is a limit to conjugation with sulphuric acid and probably the elimination of larger doses of phenol is taken over by the glucuronic acid mechanism. Figures for the distribution of phenol metabolites in the urine of rabbits have also been given by Deichmann (1944). This worker found that rabbits receiving 0·3 g. phenol/kg. orally excreted in the urine some 77% (59–88%) of the administered dose; about one-half the excreted material is free phenol and the remainder is conjugated. The conjugated phenol corresponds, therefore, to about 39% of the administered dose; about a half of this is conjugated with sulphate (i.e. 19% of the dose), a quarter with glucuronic acid[1] (i.e. *ca.* 10% of the dose) and the remainder is conjugated in forms as yet unidentified (Deichmann, 1944). Some 20% is oxidised in the rabbit.

Both phenylsulphuric and phenylglucuronic acids have been isolated from " phenol " urine. The former was isolated and synthesised by Baumann (1876*a*), and the latter (m.p. 161° and $[\alpha]_D - 82°$ in water) isolated by Külz (1883) and by Salkowski and Neuberg (1906), and synthesised by Neuberg and Niemann (1905). Both these compounds are recovered unchanged when injected into animals. Potassium phenylsulphate fed to or injected into rabbits can be recovered almost quantitatively from the urine (Rhode, 1923; Christiani, 1878). Injected phenylglucuronic acid is also mainly excreted unchanged by rabbits, but when fed by mouth the amount appearing in the urine is reduced by half and there is a rise in the excretion of ethereal sulphates (Nakano,

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[1] At dose levels of 0·1 g. per kg., the writer (Porteous and Williams, unpublished results) has found that in the rabbit about 80% of the dose is excreted in the urine; 60% is combined with glucuronic acid and 20% with sulphuric acid. The free phenol excreted is practically nil.

1937). Phenylglucuronide is therefore partially hydrolysed in the intestine, the phenol set free being partly excreted as an ethereal sulphate.

The conjugation of phenol is influenced by a number of factors such as nature of the diet, fatigue and temperature. On an acid diet (mainly oats) rabbits excrete about 30% of a given dose of injected phenol and about half of this is conjugated, but on a basic diet (potatoes and cabbage) more than 50% of the phenol is eliminated, but only about one-sixth of this is conjugated (Braunstein *et al.*, 1931). The oxidation of phenol therefore appears to be greater on an " acid " than on a " basic " diet. However, further .investigation of the effects of these diets (Braunstein and Parschin, 1931) has indicated that by altering the amount of urine excreted it is possible to induce a reversal of the behaviour of phenol excretion whilst still maintaining the acid or alkaline nature of the diet. From this it follows that the influence of different types of food upon the fate of phenol is not due to the acid or basic character of the foods, but mainly if not wholly to the alteration of water regulation which is effected by these types of foods. Fatigue (Palladin and Palladina, 1935) and abnormal body temperatures, high or low (Ito, 1916), both reduce the ability of rabbits to conjugate and oxidise phenol.

## The Sulphate Conjugation

It is generally accepted that the conjugation of phenol with sulphuric acid takes place in the liver, for Embden and Glässner (1903) showed that phenol formed ethereal sulphate in the perfused liver. However, phenol is still conjugated in the dog in the absence of the liver (Marenzi, 1931). Phenol conjugation is apparently a general function of many tissues except the kidney, but the main extra-hepatic site is the intestine. The work of Marenzi confirms the earlier work of Herter and Wakeman (1899) with tissue brei, for these workers found liver tissue and the epithelium of the intestinal tract to be the most efficient tissues for disposing of phenols. *In vitro*, intestine is more active than liver for conjugating phenol with sulphuric acid (Arnoldt and De Meio, 1941).

Studies on the metabolism of phenol in surviving slices of guinea-pig liver, show that both the disappearance and conjugation of phenol are carried out by thermolabile enzyme systems (Bernheim and Bernheim, 1943; Torda, 1943). When phenol is added to guinea-pig liver slices in Ringer-bicarbonate solution in an atmosphere of 95% $O_2$ and 5% $CO_2$ it disappears, and 20–30% of it can be accounted for by conjugation. The disappearance of phenol in guinea-pig liver is an aerobic process which is inhibited by M/300 cocaine hydrochloride, although the latter does not affect the percentage of phenol conjugated. The conjugation process does not occur anaerobically or in the absence of free sulphate ions, and cystine and methionine cannot act as sources of sulphate. In the intact cat receiving an infusion of phenol, the simultaneous infusion of cocaine lowers the output of conjugated phenol. Cocaine appears therefore to inhibit the conjugation of phenol in the living animal.

Concerning the source of sulphate for the detoxication of phenols, there has been much controversy based on conflicting experimental results. However, the balance of evidence indicates that ethereal sulphates are formed by the direct union of the sulphate ion with the phenol, and that the source of sulphate can be both endogenous and exogenous, the former source being catabolised protein and the latter administered sulphate (Hele, 1924*a* and 1931). Rhode (1923) found that the subcutaneous injection of either sodium sulphite or of cystine into rabbits receiving phenol orally, caused the output of ethereal sulphate to increase, but sodium sulphate and thiosulphate were not effective in this respect. Many other earlier workers found that injected sodium sulphate was not utilised in ethereal sulphate synthesis ; Tauber (1895), for example, found none of the following compounds, $Na_2SO_4$, $Na_2S_2O_3$, $Na_2S_2O_7$, $NaC_2H_5SO_4$, $Na_2S_2O_5$ and taurine affected the sulphate conjugation of phenol, whereas $Na_2SO_3$ increased it. On the other hand, Hele (1924*a*, 1931) found that sodium sulphate, sodium bisulphite and cystine, when given orally, could be utilised for ethereal sulphate formation from phenol, indole and guaiacol carbonate. The conflicting results of Hele and of Rhode on the utilisation of sodium sulphate for ethereal sulphate formation is explained by Hele (1924*a*) as follows. Assuming that the liver is the chief source of ethereal sulphate, sodium sulphate injected subcutaneously or intravenously is likely to be excreted by the kidney without affecting in any marked degree the concentration of sulphate in the liver cells, whereas substances like cystine or sulphite, which are not readily excreted by the kidney, may reach the liver and be oxidised actually at the site of the ethereal sulphate synthesis. This explanation is probably only half the story, in view of the work of Marenzi (1931), who showed that the intestine is an important site for ethereal sulphate synthesis. Compared with ingested sulphate injected sodium sulphate obviously cannot be used to any great extent for ethereal sulphate synthesis in the intestine. The first source of sulphur to be used for the detoxication of phenols is probably the sulphate ion of the cell fluids (Hele, 1931). As the immediate supply of sulphate is used up it is replaced by sulphate obtained by the oxidation processes of metabolism ; the latter can in part be replaced by administered sulphate.

Support for the view that exogenous sulphate can be used for ethereal sulphate formation comes from the experiments of Bernheim and Bernheim (1943), who showed that guinea-pig liver slices could conjugate phenol only in the presence of sulphate ions, for the conjugation process was abolished in the absence of free sulphate. The slices could not use cystine or methionine as sources of sulphate.

## MONOSUBSTITUTED PHENOLS

Most monosubstituted phenols are metabolised in a similar manner to phenol itself, but there are quantitative differences related to the nature and position of the substituent groups. The effect of substituents on the conjugation of phenol with sulphuric acid in the rabbit has been worked out by Williams (1938). No investigations have been recorded on the influence of substitution on the glucuronic acid conjugation or on the oxidation of phenol. The work of Williams shows that, using doses of substituted phenols equivalent to 250 mg. phenol/kg., *ortho* substituted acidic groups lower the sulphate conjugation, basic *ortho* groups increase it, whereas neutral *ortho* groups have little effect. Similar results are found with *meta* substituents, although the effects are not so marked. In the *para* position the tendency is for any group, irrespective of its nature, except the —COOH and —CONH$_2$ groups, to have little or no influence on the conjugation of phenol with sulphate (see Fig. 1). The carboxyl group in any position reduces the conjugation, the effect being greatest in the *ortho* position when the sulphate conjugation is entirely abolished, and least in the *para* position where the conjugation is less than one-half of that of unsubstituted phenol. The amide group, on the other hand, increases the sulphate conjugation of phenol by more than one-half and the effect is independent of the position of the group.

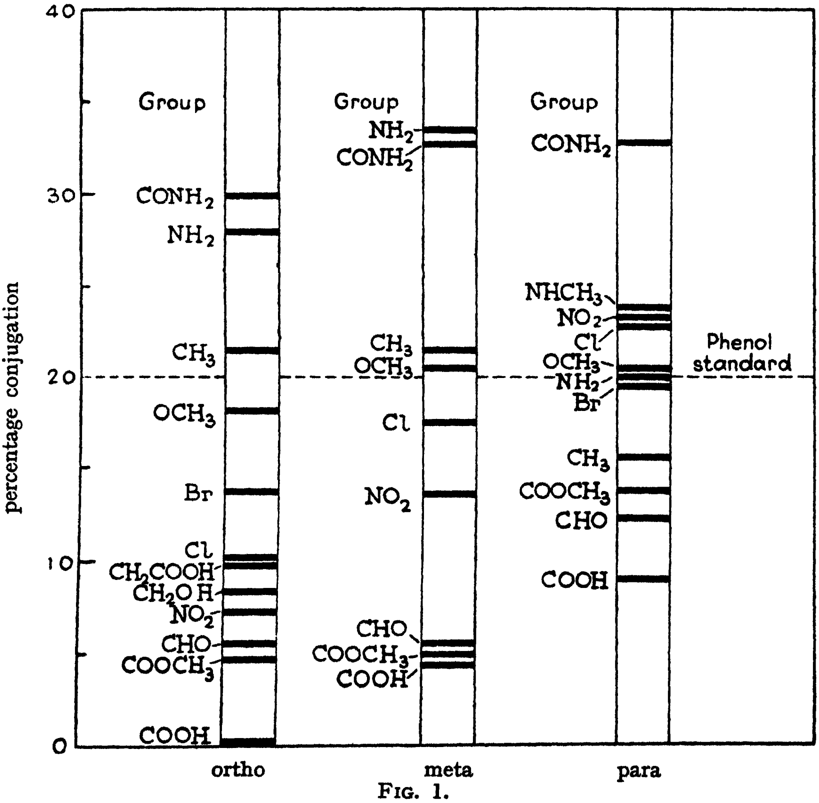
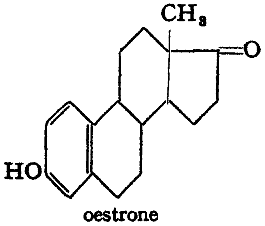


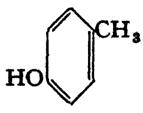
FIG. 1.

Most monosubstituted phenols are excreted partly combined with glucuronic acid but some may be oxidised to dihydric phenols. However, little quantitative information is available on these points. The following substituted phenols have received attention, mainly in reference to their ability to stimulate the excretion of ethereal sulphates ; *o*-, *m*-, *p*-chlorophenols (Külz, 1883 ; Coombs and Hele, 1926), *p*-bromophenol (Rhode, 1923), salicylamide (Baas, 1889), the cresols (see below) and guaiacol (Knapp, 1911). The hydroxybenzaldehydes, hydroxybenzoic acids, aminophenols and nitrophenols are considered elsewhere (see pp. 87, 102, 136 and 149).

**The Phenols of Normal Urine.** Phenols are excreted in normal urine and these originate from a variety of sources. A large proportion is derived from bacterial putrefaction of aromatic amino acids in the intestine, whereas others, such as sex hormones, adrenaline, etc., originate from normal metabolic processes. These phenols occur in the urine both in the free state and combined with glucuronic and sulphuric acids. Of the simpler phenols, *p*-cresol, phenol, *o*-cresol, quinol, catechol, indoxyl and *p*-hydroxybenzoic acid have been detected in urine. The daily output of phenols in the human is between 0·2 and 0·5 g., of which 30–90% may be in the free state (Folin and Denis, 1915 ; Dubin, 1916). According to Siegfried and Zimmermann (1911) *p*-cresol is quantitatively the most important phenol in normal human urine. These workers found on taking the average of nine persons, that, of the total phenols eliminated, about 58% was *p*-cresol (*ca.* 1·8 mg.%), the remainder being mainly phenol, other phenols only occurring in minute quantities. *p*-Cresol occurs in considerable amounts in horse urine and Marshall (1937) isolated approximately 110 g. of *p*-cresol free from *o*- and *m*-isomerides from the hydrolysis products of 400 gallons of urine from pregnant mares, the amount being 6 mg. %. In stallion's urine the amount of *p*-cresol excreted is even greater, amounting to 55 mg. % (Campbell and Hey, 1944). Stallion's urine is also a rich source of oestrogenic hormone (mainly oestrone) and it is possible that *p*-cresol is, in this case, derived not only from the degradation of tyrosine but also from the disruption of the steroid molecule, the benzenoid ring of oestrone surviving as *p*-cresol (cf. Hey, 1944).



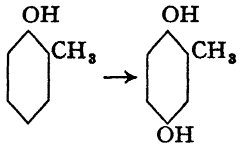
oestrone



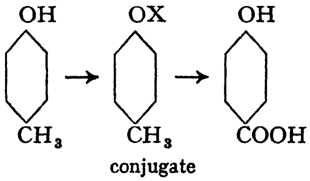
*p*-cresol

*p*-Ethylphenol has been isolated from the urine of a pregnant mare and Lederer (1943) believes this phenol to be derived from tyrosine. It is probably a normal constituent of animal urine.

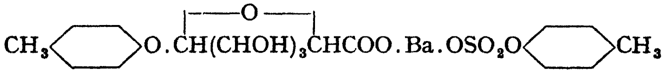
**Cresols.** All three cresols are partly eliminated by the dog as ethereal sulphates and glucuronides (Preusse, 1881) and a considerable proportion undergoes oxidation. Jonescu (1906) showed that when 1 g. of cresol was fed to a dog, 30–35% of the *ortho* compound, 47–50% of the *meta* and 25% of the *para* was eliminated, the remainder undergoing further changes. *o*-Cresol is oxidised to a slight extent to hydrotoluquinone (Preusse), thus :—



There have been no reports of it forming salicylic acid, which is a possible metabolite. *p*-Cresol is also oxidised, but in this case the product is *p*-hydroxybenzoic acid.



Both the ethereal sulphate and glucuronide of *p*-cresol have been isolated. In fact, Neuberg and Kretschmer (1911) have isolated from the urine of dogs fed with *p*-cresol a double barium of salt ($[\alpha]_D - 34{\cdot}5^\circ$) of *p*-cresylglucuronide and *p*-cresylsulphuric acid, which can probably be represented by the following formula :—

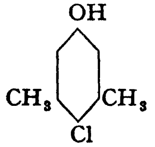


$$CH_3\langle\quad\rangle O.\overset{\vert\text{——O——}\vert}{CH(CHOH)_3CHCOO}.Ba.OSO_2O\langle\quad\rangle CH_3$$

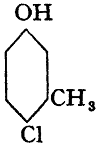
**Halogenated Phenols.** Halogenated phenols have been used in the past as external disinfectants, and it appears that they can also act as powerful internal disinfectants. According to Zondek (1942*a* ; 1942*b*), their application in the clinical treatment of pyelitis and cystitis may effect the sterilisation of the urine, and blood from treated patients is bacteriostatic.

*o*-, *m*- and ***p*-Chlorophenols** are excreted conjugated with sulphuric and glucuronic acids. In the dog, between 50 and 60% of a dose of just over one gram of these phenols is excreted as ethereal sulphates (Coombs and Hele, 1926). With larger doses, the sulphate conjugation becomes less evident and probably glucuronic acid is the main conjugation mechanism (see Fig. 1). **Bromophenols** behave similarly (Baumann, 1883 ; Rhode, 1923). There is some evidence that the monohalogenated phenols may also be converted to halogenodihydroxybenzenes (Baumann). None of the conjugates of the halogenophenols have been isolated and therefore their exact nature is obscure.

***p*-Chloro-*m*-xylenol** (4-chloro-3:5-dimethylphenol) and ***p*-chloro-*m*-cresol** (4-chloro-3-methylphenol) have versatile bacteriostatic properties and their fate in the animal body has been investigated by Zondek and Shapiro (1943). In man some 31% of injected *p*-chloro-*m*-xylenol is excreted in a combined form, 14% as a glucuronide and 17% as a sulphate. Only traces of the compound are excreted in the free state on a normal diet (slightly acid urine), but these traces are sufficient to inhibit ammoniacal fermentation: however, if the urine is made alkaline by administration of $NaHCO_3$ about 8% of free chloroxylenol is excreted and the urine is definitely bactericidal. On the other hand, the rabbit excretes 15% of injected chloroxylenol in the free state, probably because rabbit urine is normally slightly alkaline. The excretion of free chloroxylenol depends on the *p*H of the urine, and urine with bactericidal properties is favoured by a high *p*H such as that produced after $NaHCO_3$ administration. Urine rendered acid by administration of $NH_4Cl$ results in the cessation of the excretion of free chloroxylenol. In the rat, 48 hours after injection with chloroxylenol, some 30% (free and combined) is excreted in the urine, 15% appears in the faeces and about 10% remains in the carcase. The rest is changed to other compounds of undetermined nature. The related *p*-chloro-*m*-cresol is not excreted in the free state by man even after alkalisation, although the rabbit excretes some 15–20%.



chloroxylenol



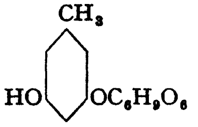
chlorocreso

## POLYHYDRIC PHENOLS

As regards their conjugation, polyhydric phenols behave in a similar manner to phenol, forming ethereal sulphates and glucuronides. But in most cases only one of the phenolic hydroxyls is conjugated and ethereal sulphates and glucuronides are excreted containing free phenolic hydroxyl groups. There are probably only a few phenols in which more than one OH may be conjugated. Our information regarding the metabolism of the polyhydric phenols is, however, somewhat scanty.

**The Dihydric Phenols.** Little is known regarding the fate of **catechol, resorcinol** and **quinol** in the animal body. All three form ethereal sulphates and glucuronides, but generally the nature of these compounds is obscure (Baumann). Resorcinol is said to form a mono- and a di-ethereal sulphate (Baumann and Herter, 1877–8), and resorcinylglucuronide has been prepared by Külz (1890). Quinol forms an ethereal sulphate and is partly oxidised to quinone. There is evidence to show that quinol exerts its toxic effects through the redox system quinol-quinone (Labes, 1929; Oettel, 1936).

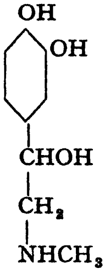
**Orcinol** (3:5-dihydroxytoluene) forms both an ethereal sulphate (Baumann and Herter, 1877–8) and a monoglucuronide; the latter has been isolated as a barium salt ($[\alpha]_D - 73{\cdot}6°$) from the urine of rabbits receiving orcinol (Sera, 1913; 1914).



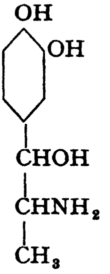
orcinylglucuronide

**Resacetophenone** (2:4-dihydroxyacetophenone) forms a mono-ethereal sulphate and a monoglucuronide in the dog. Both these compounds were isolated by Nencki (1894), the sulphate as a potassium salt, $C_6H_3(COCH_3)(OH)(OSO_3K)$, and the glucuronide both in the free state (decomp .170°) and as a copper salt, $C_{14}H_{14}O_9Cu, 4H_2O$. Which one of the two hydroxyl groups is conjugated is unknown.

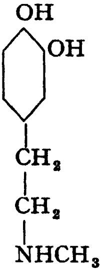
**Adrenaline.** Adrenaline and the related compounds **epinine** and *dl*-**corbasil**, are excreted by the human being in a conjugated form (Richter, 1940).



adrenaline



corbasil



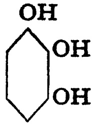
epinine

In the case of *d*-adrenaline some 71% of a dose of 55 mg. is excreted in a combined form. Adrenaline is excreted as a sulphuric ester which may be adrenaline 3- or 4-hydrogen sulphate, a compound which would normally exist as a " zwitterion ",

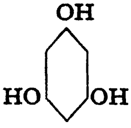
$$\overset{-}{SO_3}.O(OH)C_6H_3CHOH.CH_2\overset{+}{N}H_2CH_3$$

Neither *d*- nor *l*-adrenaline is oxidised to protocatechuic acid as might be expected. Richter points out that phenylethylamine and most other amines are oxidised, and hence inactivated, by amine oxidase. In the case of adrenaline, conjugation is more rapid than oxidation by amine oxidase, and this suggests that conjugation is probably the main physiological method by which adrenaline is inactivated in the body.

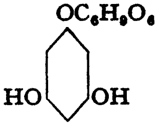
**Trihydric Phenols.** Both **pyrogallol** (1:2:3-trihydroxybenzene) (Baumann and Herter, 1877) and **phloroglucinol** (1:3:5-trihydroxybenzene) form ethereal sulphates in the animal. In the case of phloroglucinol, the monoglucuronide has been isolated from the urine of rabbits as a potassium salt ($[\alpha]_D - 80{\cdot}8°$) (Sera, 1914). **Gallacetophenone** (2:3:4-trihydroxyacetophenone) behaves similarly (Nencki, 1894).



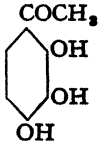
pyrogallol



phloroglucinol



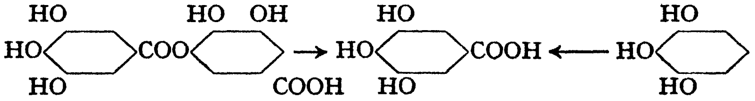
phloroglucinyl-glucuronide



gallacetophenone

**Tannic Acid.** In 1848 Wöhler and Frerichs found that when tannic acid was fed to a dog gallic acid and probably pyrogallol were excreted in the urine. Baumann and Herter (1877–8), however, did not find pyrogallol, but isolated crystalline gallic acid from dog urine. These workers also noted that there was no rise in the ethereal sulphate output of tannic acid-fed dogs. Later Baumann (1882) was able to isolate small amounts of crystalline gallic acid from normal horse urine and he expressed the opinion that the acid originated from tannin material present in the animal's food. Subsequent workers (Mörner, 1892; Harnack, 1898) found that tannic acid was also excreted as gallic acid by human beings, but they did not find pyrogallol. Preparations of tannic acid such as tannigen (diacetyl tannic acid) and tannalbin (a tannic acid-albumin complex) which are used in medicine also yield gallic acid in the urine when administered to man, dogs and rabbits (Rost, 1897). Rost has further suggested that tannic acid yields other metabolites, including probably pyrogallol, in addition to gallic acid, but the exact nature of these is not clear. No tannic acid is excreted unchanged. Gallic acid is less toxic to mice than tannic acid (Robinson and Graessle, 1943).

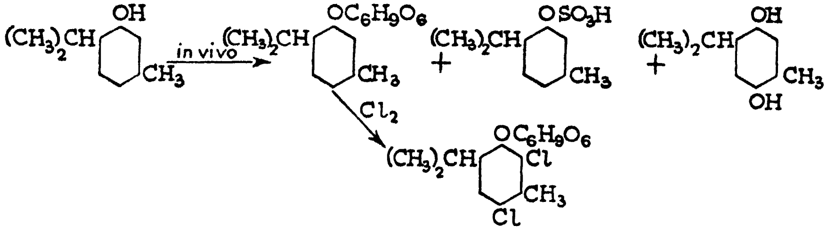
It appears, therefore, that tannic acid is split *in vivo* to two molecules of gallic acid which may then be partly decarboxylated to pyrogallol. If the latter substance were formed it could be expected to be partly excreted as an ethereal sulphate (see p. 76).



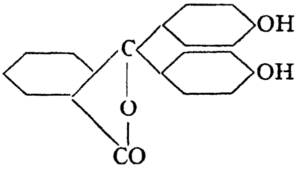
tannic acid

## MISCELLANEOUS PHENOLS

**Thymol** (3-methyl-6-*iso*propylphenol). This phenol has been widely used for the treatment of infections by intestinal parasites. The products of its metabolism in man are stated to be thymolglucuronide, thymol ethereal sulphate and the ethereal sulphate of thymohydroquinone. The glucuronide was isolated by Blum (1892) in a rather interesting manner. Urine from patients receiving thymol was filtered and treated with three times its volume of concentrated hydrochloric acid. Sodium hypochlorite was then added and on standing dichlorothymolglucuronide separated as large needles. The same compound can be obtained by a similar treatment of urine from thymol-treated dogs and rabbits (Katsuyama and Hata, 1898; Takao, 1923). The acid melts at 125–126° and shows $[\alpha]_D - 66{\cdot}5°$. Blum formulated it as 2:4-dichloro-3-methyl-6-*iso*-propylphenylglucuronide.



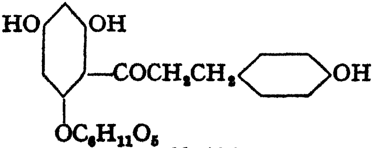
**Phenolphthalein.** The discovery that phenolphthalein has a purgative action on the organism stimulated an interest in its metabolism. Phenolphthalein has two phenolic hydroxyl groups, but they are attached to different aromatic rings and only one of the hydroxyls undergoes conjugation *in vivo*. Phenolphthalein and its tetrachloro derivative, phenoltetrachlorophthalein, are excreted by dogs as conjugated sulphates



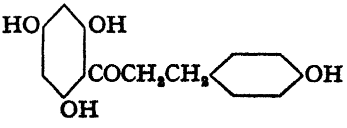
phenolphthalein

and glucuronides (Fleig, 1909; Abel and Rowntree, 1909). In the human, small doses (30–60 mg.) are excreted in a conjugated form, but with larger doses (300 mg.) free as well as combined phenolphthalein are excreted. There is no doubt that a large proportion of phenolphthalein is degraded in the body, for Di Somma (1940) found that, at best, only 45% of injected phenolphthalein could be recovered from the urine of rabbits. Di Somma also isolated from rabbit urine phenolphthalein monoglucuronide as a cinchonidine salt which crystallises with one molecule of alcohol (or of dioxan) of crystallisation (depending upon the solvent from which it is crystallised). The alcoholate, $C_{45}H_{44}O_{11}N_2$, $C_2H_5OH$ is laevorotatory, showing $[\alpha]_D - 64{\cdot}5°$ (in 80% alcohol).

**Phloridzin.**[1] This phenolic glucoside has been of the greatest value in elucidating the problems of carbohydrate metabolism. It is obtained from the bark of fruit trees and, particularly, from the root cortex of the apple-tree. Chemically it is the 2-glucoside of phloretin and possesses three free phenolic hydroxyl groups (Müller and Robertson, 1933) :—



**phloridzin**



**phloretin**

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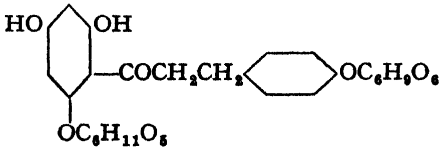
[1] Also given as phlorrhizin and phlorhizin.

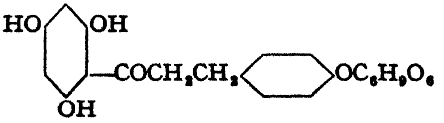
When phloridzin (or its aglucone, phloretin) is administered orally or subcutaneously it gives rise to an intense glycosuria without an accompanying hyperglycaemia; in fact, the blood-sugar is diminished and the renal threshold for sugar is abolished. Its action is said to be due to the fact that it poisons the enzyme phosphatase which is concerned with the synthesis of hexosephosphate in the kidney. Glucose is not reabsorbed as such but as hexosephosphate; the kidney tubules therefore, in the presence of phloridzin, allow sugar to escape into the urine instead of returning it to the blood as in the normal kidney (Lundsgaard, 1933). The metabolism of phloridzin in the animal body should therefore be of great interest.

Von Mering (1889), the discoverer of phloridzin diabetes, had found that a part of subcutaneously injected phloridzin was excreted unchanged in the urine for the latter gave a positive ferric chloride reaction. Yokotas (1904) accounted for 92% of injected phloridzin, by hydrolysing phloridzin-containing material isolated from urine, and weighing the resulting phloretin. Cremer (cf. Schüller, 1911) then discovered that urine from phloridzinised animals after removal of the glucose by fermentation was still laevorotatory and contained a substance which could be extracted with ethyl acetate. This substance was eventually isolated from the urine of rabbits receiving phloridzin by injection by Schüller (1911), and shown to be phloridzin glucuronic acid, a crystalline substance ($[\alpha]_D$ — 102·3° in water) giving a brown-red colour with ferric chloride. On partial hydrolysis with dilute sulphuric acid, it gave glucose and phloretin glucuronic acid ($[\alpha]_D$ — 68·7° in water). This fact showed that the original glucose molecule of phloridzin was still present in phloridzin glucuronide which therefore contained both glucose and glucuronic acid. Hydrolysis of phloretinglucuronide gave crystalline phloretin and glucuronic acid, the latter being identified by the naphthoresorcinol reaction and the formation of furfural when distilled with hydrochloric acid.

The position of attachment of glucuronic acid to the phloridzin molecule is uncertain, but Schüller suggests without experimental evidence, that it is attached to the phenol ring and not to either of the phloroglucinol hydroxyl groups. Schüller's formulae are given below, using the corrected structure for phloridzin (Müller and Robertson, 1933).



phloridzinglucuronide

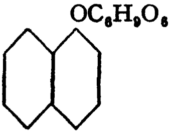


phloretinglucuronide

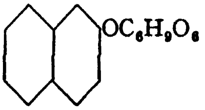
In addition to phloridzinglucuronide, small amounts of other laevorotatory metabolites of unknown composition are excreted, and Schüller (1911) suggests that the ethereal sulphate of phloretin may also be present.

By conjugating with glucuronic acid, the effect of phloridzin on the excretion of sugar is altered, for whereas 1·3 g. of phloridzinglucuronide injected into a dog will cause the excretion of 3–5 g. of glucose in 24 hours, the equivalent amount of phloridzin causes the excretion of 20 g. or more (Schüller, 1911). The formation of phloridzinglucuronide thus results in a partial detoxication of phloridzin and the action of phloridzin on the kidney must be due mainly to the unconjugated glucoside.

**The Naphthols.** **β-Naphthol** was employed for many years in the treatment of hookworm disease, although it has now been replaced by other and more efficient anthelminthics. Both α- and β-naphthols are converted in the body to ethereal sulphates and glucuronides, and are probably oxidised to a slight extent to dihydroxynaphthalenes (Mauthner, 1881; Lesnik and Nencki, 1886). The glucuronides both of α- and β-naphthols have been isolated from the urine of animals receiving these phenols. The behaviour of the naphthols in the animal body is therefore qualitatively similar to that of phenol. α-Naphthyl-β-glucuronide is a crystalline solid (m.p. 202–203°, $[\alpha]_D - 89{\cdot}3°$) and a product of the metabolism of naphthalene. β-Naphthyl-β-glucuronide is a hydrated crystalline solid (m.p. 100°; $[\alpha]_D - 90{\cdot}65°$; in the anhydrous state it melts at 150° and shows $[\alpha]_D - 100{\cdot}8°$) (Lesnik and Nencki; Bergmann, 1933; Umezawa and Masamune, 1938).



α-naphthylglucuronide



β-naphthylglucuronide

## *Chapter Six*

# The Metabolism of Aromatic Alcohols, Ethers, Aldehydes, Ketones and Amides

### Aromatic Alcohols and their Esters

IN general, *primary aromatic alcohols* appear to undergo oxidation in the animal body with the production of either benzoic acid or phenylacetic acid according to the length of the side-chain. Thus benzyl alcohol is converted to benzoic acid, whereas phenylethyl alcohol produces phenylacetic acid (Neubauer, 1901). Substituted aromatic alcohols behave similarly for saligenin (*o*-hydroxybenzyl alcohol) is converted to salicylic acid *in vivo* (Nencki, 1870). There is no direct evidence available to show that they form glucuronides, although this is probable in some cases, since the *o*-nitrobenzyl alcohol formed during the metabolism of *o*-nitrotoluene in the dog is excreted as *o*-nitrobenzylglucuronide (Jaffe, 1878–9).

Little is known regarding the fate of *secondary aromatic alcohols* in the body, but we do know something of their metabolism since they are formed *in vivo* during the metabolism of aromatic ketones (cf. acetophenone, etc., p. 86). Since the secondary aromatic alcohols possess a branched chain, they are likely to be oxidised with more difficulty than the primary. A large amount of the secondary alcohols formed during the reduction of ketones are excreted as conjugated glucuronides, for in the case of acetophenone some 36% of it is excreted by the rabbit as phenylmethylcarbinol glucuronide.

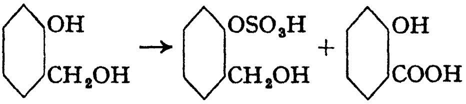
The only *tertiary aromatic alcohol* that has been investigated appears to be **triphenylcarbinol** $(C_6H_5)_3COH$, and Miriam, Wolf and Sherwin (1927) have shown that when fed to rabbits it is mainly excreted unchanged in the urine and faeces.

Esters of aromatic alcohols appear to follow the same fate as the free alcohols. The esters are presumably first hydrolysed to the free alcohols which are then metabolised to the corresponding acids.

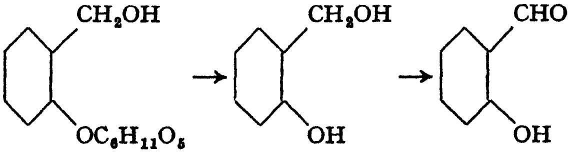
**Benzyl Alcohol.** Benzyl alcohol and its esters possess anti-spasmodic activity (Macht, 1918) and have been used in the treatment of such conditions as asthma and angina pectoris. The activity of benzyl esters in this respect has been shown to be due to the action of the benzyl alcohol liberated by hydrolysis. The relative efficiency of benzyl esters as spasmolytics is therefore dependent on their rate of hydrolysis *in vivo* and that of benzyl alcohol upon its rate of oxidation. The antispasmodic activity and the metabolism of the benzyl alcohol derivatives are therefore closely related.

Schmiedeberg (1881) first discovered that benzyl alcohol was oxidised *in vivo* to benzoic acid, and the investigations of Snapper, Grünbaum and Sturkop (1924–5) have shown that the alcohol is very rapidly oxidised in man and some 80–90% can be accounted for by the hippuric acid excreted in the urine. In fact, both in man and in the rabbit the rate of excretion of hippuric acid after benzyl alcohol is almost as rapid as after sodium benzoate (Snapper *et al.*, 1924–5; Diack and Lewis, 1928), for Diack and Lewis found 69·5% of sodium benzoate and 65·7% of benzyl alcohol to be excreted by the rabbit as hippuric acid in 6 hours. It appears that the rate of oxidation of benzyl alcohol is at least as great as the rate of hippuric acid synthesis and excretion. Snapper *et al.* also found orally administered benzyl acetate, benzoate, cinnamate and hydrocinnamate to be rapidly hydrolysed and oxidised, and Diack and Lewis found monobenzyl and dibenzyl succinate to be more slowly eliminated probably due to a slower rate of hydrolysis and absorption from the intestine.

**Saligenin.** This phenolic alcohol which possesses limited antispasmodic properties (Dunning, Dunning and Reid, 1936) is converted *in vivo* to salicylic acid. In the rabbit it causes an increased output of ethereal sulphate corresponding to 6–10% of the saligenin ingested. Since salicylic acid itself does not form an ethereal sulphate in the rabbit, conjugation of saligenin with sulphuric acid must take place to some extent before the $-CH_2OH$ group is oxidised (Williams, 1938).



In the larvae of the brassy willow beetle (*Phyllodecta vitellinae*) it appears that saligenin is oxidised to salicylaldehyde. These larvae live on willow leaves which contain the glucoside, salicin (saligenin glucoside), and when disturbed they secrete from certain dorsal glands a strongly odorous fluid containing salicylaldehyde (Wain, 1943). The secretion which is not produced by the adult beetles, appears to serve as a protective mechanism. According to Wain, salicin is probably converted to salicylaldehyde as follows:—



In **cinnamic alcohol**, $C_6H_5CH{:}CHCH_2OH$, the double bond is apparently hydrogenated by the rabbit, but in the α-ethyl derivative, $C_6H_5CH{:}C(C_2H_5)CH_2OH$, the double bond is unchanged and about 30–33% is converted into α-ethylcinnamic acid (Fischer and Bielig, 1940).

$$C_6H_5CH{:}CHCH_2OH \rightarrow C_6H_5CH_2CH_2COOH \rightarrow C_6H_5COOH$$

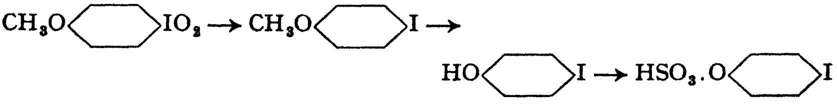
$$C_6H_5CH{:}C(C_2H_5)CH_2OH \rightarrow C_6H_5CH{:}C(C_2H_5)COOH$$

## AROMATIC ETHERS

Aromatic ethers may undergo two changes in the body, the first involving oxidation of the aromatic ring, thereby producing a phenolic ether, and the second involving the ether linkage which is split with the production of a phenol. A combination of both may also occur with the production of a dihydric phenol. Oxidation of the ring normally takes place in the position *para*—if this is free—to the ether linkage.

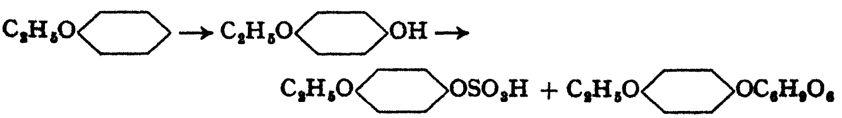
(*a*) ArOX → *p*-HOArOX
(*b*) ArOX → ArOH
(*c*) ArOX → *p*-HOArOH
Ar = aryl group. X = alkyl or aryl group.

**Anisole** (phenylmethyl ether). During an investigation on the synthesis of mercapturic acids in animals, Coombs and Hele (1926) fed anisole and its *m*-chloro, *o*- and *p*-bromo derivatives to dogs. They found large increases in the ethereal sulphate output corresponding to 30% of the dose in the case of anisole, and to 40–50% of the dose in the case of its halogen derivatives. It appears probable in these cases that the ether link is split and the resulting phenol conjugated with sulphuric acid. This explanation is supported by the fact that the ether linkage is known to be split in the case of ***p*-iodoxyanisole** (an antiseptic known as " isoform "). When this compound is fed to dogs there is excreted the ethereal sulphate of iodophenol. Röhmann (1905) represents the metabolism of iodoxyanisole as follows :—

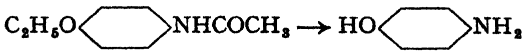


It should be noted that the iodoxy group is reduced *in vivo* to an iodo group (see iodosobenzene, p. 65).

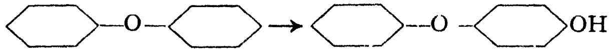
**Phenetole** (phenylethyl ether) was studied by Kossel (1880, 1883) who, after feeding it to dogs isolated from the urine a conjugated glucuronic acid, " quinethonic acid " (m.p. 146° and $[\alpha]_D - 63°$). Kossel showed that phenetole was probably oxidised *in vivo* to *p*-ethoxyphenol, since quinethonic acid on heating with manganese dioxide and sulphuric acid yields quinone. The structure of quinethonic acid (*p*-ethoxyphenylglucuronide) was determined by Lehmann (1889), who isolated *p*-ethoxyphenol (*p*-hydroxyphenetole) from its hydrolysis products. The ethereal sulphate of *p*-ethoxyphenol after feeding phenetole has also been isolated (Kühling, 1887). The metabolism of phenetole can therefore be represented as follows :—



Closely related to phenetole is the coal-tar antipyretic **phenacetin** (*p*-ethoxyacetanilide). *In vivo* the ether linkage of this compound is split and *p*-aminophenol is formed (see p. 149).

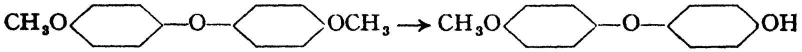


**Diphenyl ether.** In rabbits diphenyl ether is converted to 4-hydroxydiphenyl ether to the extent of 22·8% ; the latter is excreted mainly in the free state (20·7%), although a small proportion (1·6%) is combined (Stroud, 1940*a*).

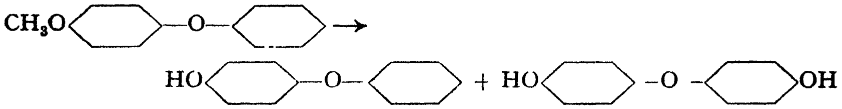


None of the injected diphenyl ether is excreted unchanged. Whereas diphenyl ether itself is oestrogenically inactive, its phenolic metabolic product is slightly active and 100 mg. will produce full oestrous response when injected into ovariectomised rats.

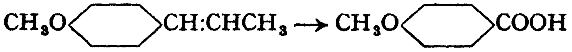
**4:4′-Dimethoxydiphenyl ether** has three ether linkages, but apparently only one is ruptured *in vivo.* Stroud found 4-hydroxy-4′-methoxydiphenyl ether, both free and combined, in the urine of rabbits injected with the 4:4′-dimethoxy ether.



**4-Methoxydiphenyl ether** undergoes both oxidation and demethylation when injected into rabbits, for 4:4′-dihydroxy- and 4-hydroxydiphenyl ethers are found in the urine.



In the allyl ethers, **anethol** and **eugenol,** the allyl group is oxidised to carboxyl. Some eugenol is excreted as an ethereal sulphate (Kühling and Giacosa, 1890). Whether the ether link is split or not, is not known.



anethol anisic acid

**Thioethers. Dibenzyl thioether** is converted by the rat into hippuric acid :—

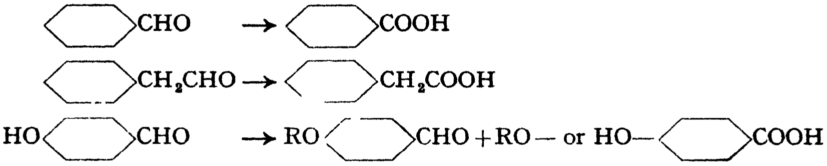
$$C_6H_5CH_2SCH_2C_6H_5 \rightarrow C_6H_5CONHCH_2COOH$$

Although a mercapturic acid is a possible metabolite (cf. benzyl chloride, p. 61), no trace of such a compound is produced (Stekol, 1941).

## AROMATIC ALDEHYDES

In the animal organism at least three enzymes are found which can catalyse reactions with aldehydes. Aldehyde mutase catalyses the dismutation of two molecules of aldehyde with the formation of a molecule of alcohol and one of acid. This enzyme's activity is confined mainly to the lower aliphatic aldehydes, for the aromatic aldehydes are only very slowly attacked, if at all. The enzyme can be obtained from liver and is a protein with a pyridine-nucleotide prosthetic group (Dixon, 1939). The other two enzymes are flavoproteins, one being the well-known Schardinger enzyme of milk (xanthine oxidase). The oxidation of aldehydes in the liver is catalysed by xanthine-aldehyde oxidase and a specific aldehyde oxidase (see Green, 1941), but whereas the former enzyme acts on hypoxanthine the latter shows no activity towards this substrate. These flavin enzymes oxidise both aliphatic and aromatic aldehydes to the corresponding acids and can catalyse direct oxidation by means of molecular oxygen.

Aromatic aldehydes are oxidised *in vivo* almost completely to aromatic acids. A few cases are, however, known in which some of the aldehyde may be excreted unoxidised, and these are usually phenolic aldehydes. Phenylacetaldehyde derivatives are oxidised to phenylacetic acid derivatives.



$(R = -SO_3H$ or $-C_6H_9O_6)$

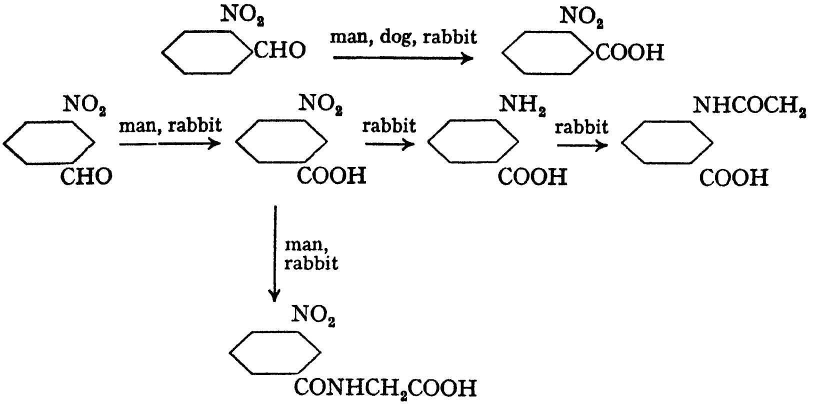
**Benzaldehyde.** Wöhler and Frerichs (1848) first discovered that benzaldehyde on injection into animals was oxidised to benzoic acid and excreted as hippuric acid (see also Cohn, 1890, 1893; Friedmann and Turk, 1913). Cohn also claimed that some of the aldehyde is excreted as benzamide and some as cinnamic acid. However, the researches of Friedmann and Turk showed that the only products of benzaldehyde metabolism in dogs and rabbits are benzoic acid and hippuric acid, for in one experiment where 10 g. of benzaldehyde was fed to a dog, 12·18 g. of hippuric acid and 0·21 g. of benzoic acid were isolated from the urine. The oxidation of benzaldehyde to benzoic acid is independent of the acidic or basic character of the diet, although an acid diet tends to decrease the excretion of benzoic acid as hippuric acid (Zuverkalov, 1930).

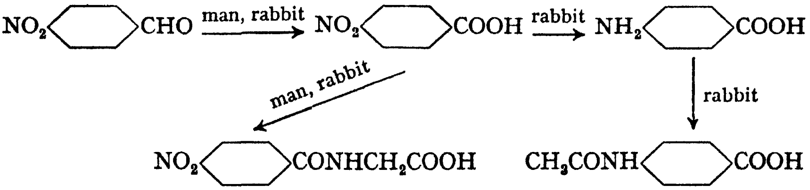
## SUBSTITUTED BENZALDEHYDES

Substituted benzaldehydes are metabolised in the animal body in a manner qualitatively similar to the parent substance. They are oxidised to the corresponding aromatic acids which are excreted partly free, and partly combined with glycine or glucuronic acid. From a quantitative standpoint their fates are, however, influenced by the nature and the position of the substituent groups.

### Nitrobenzaldehydes

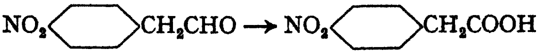
*o*-**Nitrobenzaldehyde** is oxidised in the rabbit to *o*-nitrobenzoic acid and is excreted as such. *m*-**Nitrobenzaldehyde,** however, undergoes changes which include oxidation, reduction, acetylation and glycine conjugation so that *m*-nitrobenzoic, *m*-nitrohippuric and *m*-acetaminobenzoic acids are excreted. ***p*-Nitrobenzaldehyde** is converted to *p*-nitrobenzoic acid, *p*-acetaminobenzoic acid and a compound of these two acids in equimolecular proportions (Cohn, 1893, 1894). The latter compound is probably an artefact.



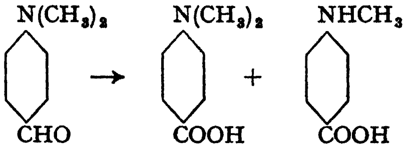


When *o*-nitrobenzaldehyde is fed to rabbits only about 10% can be recovered as *o*-nitrobenzoic acid, whereas in man and in the dog the recovery is relatively large (80%) (Sherwin and Hynes, 1921). Both *m*- and *p*-nitrobenzaldehydes are excreted to a considerable extent (90%) by man as the corresponding nitrobenzoic and nitrohippuric acids. The nitrohippuric acids constitute about a quarter of the total acids excreted. Although reduction of the nitro group to amino takes place in rabbits it does not in man (Sherwin and Hynes).

***p*-Nitrophenacetaldehyde** is merely oxidised to *p*-nitrophenylacetic acid and excreted as such without conjugation in man, the rabbit and the dog (Sherwin and Hynes).



***p*-Dimethylaminbenzaldehyde** is not only oxidised to the corresponding acid but also partly demethylated, for when fed to rabbits both *p*-dimethylaminobenzoic and *p*-monomethylaminobenzoic acids can be isolated from the urine (Jaffe, 1905). Both these acids are excreted as ester glucuronides.



## PHENOLIC ALDEHYDES

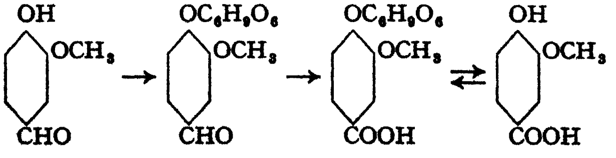
Aromatic aldehydes containing a phenolic group are of special interest since they reveal a tendency of the aldehyde group to be excreted unoxidised. Aldehydes containing a hydroxyl group in the *para* position (and probably those with an *ortho* hydroxyl) are only partly oxidised to the corresponding acid. Some of the hydroxyaldehyde is excreted as a glucuronosidobenzaldehyde (see Sammons and Williams, 1941). It appears that the presence of a phenolic hydroxyl group tends to hinder the oxidation of the aldehyde group.

*p*-**Hydroxybenzaldehyde** affords a good illustration. In the dog this aldehyde is excreted as *p*-glucuronosidobenzoylglucuronide, *p*-hydroxyhippuric acid and free *p*-hydroxybenzoic acid (Quick, 1932; Dakin, 1910). It appears probable that conjugation of the phenolic hydroxyl with glucuronic acid precedes oxidation of the aldehyde group. In the case of rabbits, *p*-glucuronosidobenzaldehyde can be isolated from the urine as a 2:4-dinitrophenylhydrazone (Williams, unpublished) and its metabolism can probably be represented as follows:

$$HOC_6H_4CHO \rightarrow C_6H_9O_6.OC_6H_4CHO \rightarrow C_6H_9O_6.OC_6H_4COOH \rightarrow$$
$$C_6H_9O_6.OC_6H_4CO.OC_6H_9O_6$$

In the case of salicylaldehyde and 2:4-dihydroxybenzaldehyde some of the free aldehyde is excreted unchanged by the rabbit, whereas protocatechuic aldehyde (3:4-dihydroxybenzaldehyde) is excreted partly as a glucuronosidobenzaldehyde. The main metabolite of salicyaldehyde in the cat, dog and rabbit is salicylic acid (Dakin, 1910; Wöhler and Frerich, 1848), but in human beings it has been said to be mainly excreted unchanged (Baumgarten, 1906), although this appears to the writer to be unlikely.

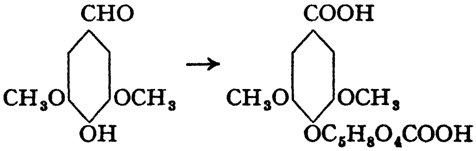
**Vanillin** (3-methoxy-4-hydroxybenzaldehyde) behaves as a typical phenolic aldehyde since it is partly excreted as glucurovanillin. Sammons and Williams (1941) have shown that for vanillin fed at a dose level of 1 g./kg. some 69% is oxidised in the rabbit to vanillic acid derivatives both free and combined, whereas 14% is excreted as unoxidised but conjugated vanillin. Vanillin gives rise to two glucuronides in the rabbit, namely, glucurovanillic acid and glucurovanillin, the latter no doubt being the precursor of the former.



**vanillin** **glucurovanillin** **glucurovanillic acid** **vanillic acid**

Some 8% of the vanillin can be accounted for as ethereal sulphates probably of vanillic acid.

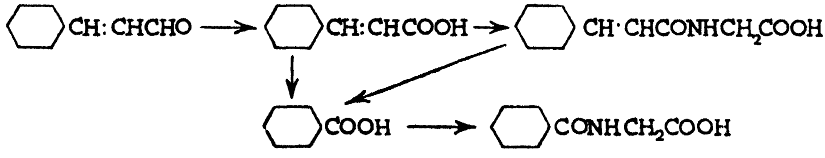
**Syringa aldehyde** (3:5-dimethoxy-4-hydroxybenzaldehyde) is converted by rabbits into a glucuronide with two carboxyl groups which is probably 3:5-dimethoxy-4-glucuronosidobenzoic acid, the glucuronide of syringic acid (Hildebrandt, 1906).

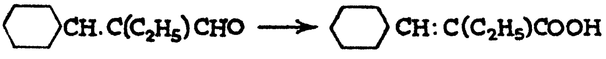


## MISCELLANEOUS ALDEHYDES

The following aldehydes have been shown to be oxidised to the corresponding aromatic acids, ***p*-tolualdehyde, anisaldehyde** (*p*-methoxybenzaldehyde), **veratraldehyde** (3:4-dimethoxybenzaldehyde), **piperonal** (3:4-methylenedioxybenzaldehyde), *o*- and ***p*-aminobenzaldehyde**. All these aldehydes give rise to ester glucuronides in the rabbit (Sammons and Williams, 1946; Heffter, 1895; Ellinger and Hensel, 1914; Hosoda, 1926).

Of the unsaturated aldehydes, **cinnamic** and **α-ethylcinnamic aldehydes** have been investigated. The former is oxidised *in vivo* to cinnamic acid, which is then degraded to benzoic acid (Friedmann and Mai, 1931), whereas the latter is oxidised to α-ethylcinnamic acid, which is not further metabolised (Fischer and Bielig, 1940).





## AROMATIC KETONES

Most ketones have some hypnotic activity, although true aromatic ketones have only a mild action. Mixed ketones such as acetophenone (hypnone) and phenylethyl ketone are much more active. Benzophenone and some of its derivatives, particularly 2:4-dichlorobenzophenone, are believed to possess bacteriostatic activity *in vitro* against human tubercle bacilli (Freedlander, 1942).

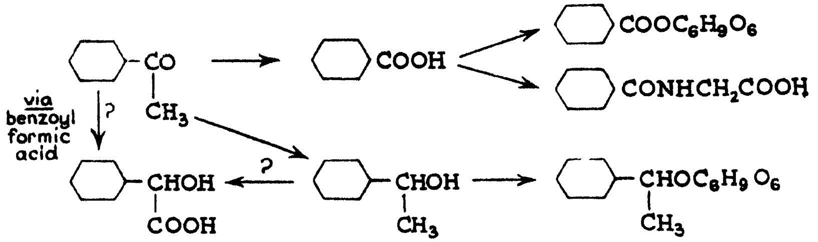
In the animal body, the carbonyl group of a mixed ketone is reduced to a secondary alcohol which is usually excreted combined with glucuronic acid. Mixed ketones are also partly oxidised to aromatic acids and, according to Dakin, if the side-chain to which the keto group is attached contains an odd number of carbon atoms, benzoic acid is formed, whereas phenylacetic acid is produced if the side-chain contains an even number. Dakin (1908) showed that benzylmethylketone, $C_6H_5CH_2COCH_3$, formed benzoic acid *in vivo*, whereas phenylethyl methyl ketone, $C_6H_5CH_2CH_2COCH_3$ (Dakin, 1909), and phenylbutyl methyl ketone, $C_6H_5CH_2CH_2CH_2CH_2COCH_3$ (Hermanns, 1913), formed phenylacetic acid. On the other hand, Thierfelder and Daiber (1923) claim that all mixed ketones give benzoic acid in the body, irrespective of whether the keto group is attached to the ring or is in the side-chain, and that the number of carbon atoms in the alkyl group has no influence on the oxidation. Only in one case, that of benzyl *iso*propyl ketone, $C_6H_5CH_2COCH(CH_3)_2$, did they find phenaceturic acid in the urine, and this occurred only in very small amounts. It may be that the oxidation of mixed ketones *in vivo* is more complex than indicated above and may depend on the position of the keto group as in the case of the $\alpha$-, $\beta$- and $\gamma$-keto-$\omega$-phenyl acids (see p. 116). Acetophenone, which has an even number of carbon atoms in the side-chain, yields benzoic acid and not phenylacetic acid, as would be expected if Dakin's statements are accepted. It is obvious that further investigations are necessary to clear up these inconsistencies. The results obtained by Dakin, Hermanns and Thierfelder are summarised in the following table :—

TABLE 8

*End Products of the Biological Oxidation of Mixed Ketones*

| Ketone | Formula | End Product | Author |
|---|---|---|---|
| Phenyl methyl | $Ph.CO.CH_3$ | Benzoic acid | Thierfelder and Daiber (1923) |
| Phenyl ethyl | $Ph.CO.CH_2CH_3$ | „ „ | Thierfelder and Daiber (1923) |
| Benzyl methyl | $PhCH_2.CO.CH_3$ | „ „ | Dakin (1908) ; Hermanns (1913) |
| Phenyl *n*-propyl | $Ph.CO.CH_2CH_2CH_3$ | „ „ | Thierfelder and Daiber (1923) |
| Phenylethyl methyl | $PhCH_2CH_2.CO.CH_3$ | Phenylacetic acid | Dakin (1909) |
| Benzyl *n*-propyl | $PhCH_2.CO.CH_2CH_2CH_3$ | Benzoic acid | Thierfelder and Daiber (1923) |
| Benzyl *iso*propyl | $PhCH_2.CO.CH(CH_3)_2$ | Benzoic acid and some phenylacetic acid | Thierfelder and Daiber (1923) |
| Phenyl-*n*-butyl methyl | $PhCH_2CH_2CH_2CH_2.CO.CH_3$ | Phenylacetic acid | Hermanns (1913) |

**Acetophenone.** Nencki in 1878 showed that when acetophenone is administered to dogs it is changed to benzoic acid and excreted as hippuric acid. Later workers (Sundwick, 1886 ; Neubauer, 1901) showed that it also formed a glucuronide both in dogs and in rabbits. A more thorough study was made by Thierfelder and Daiber (1923) by injecting the ketone subcutaneously into rabbits. They found 35·7% to be reduced to phenylmethylcarbinol which was excreted as a glucuronide, and 24·3% to be oxidised to benzoic acid and excreted as hippuric acid. Small amounts of mandelic acid, free phenylmethylcarbinol and unchanged ketone were also excreted, and some 40% was not accounted for. Phenylmethylcarbinol glucuronide was isolated as a crystalline potassium salt ($[\alpha]_D - 124{\cdot}4^\circ$) ; on hydrolysis the salt gives the free carbinol possessing a small dextro-rotation ($[\alpha]_D + 0{\cdot}7^\circ$ ; the pure (+) form has $[\alpha[_D + 42{\cdot}9^\circ$). According to Quick (1928) some of the metabolic benzoic acid derived from acetophenone is excreted as benzoylglucuronide. The fate of acetophenone can be represented as follows :—



The sequence in which the metabolites are formed is not quite clear.

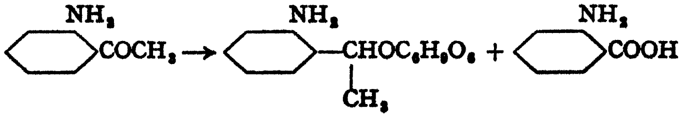
The approximate extent to which some ketones are reduced to carbinols is given in the following table (cf. Thierfelder and Daiber, 1923).

TABLE 9

*The Biological Reduction of Ketones to Carbinols*

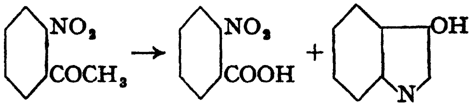
| Ketone | Percentage reduced to carbinol | Rotation of glucuronide in water, $[\alpha]_D$ |
|---|---|---|
| Acetophenone | 35·7, as phenylmethyl carbinol | − 124·4° (K salt) |
| Propiophenone | 26, as phenylethyl carbinol | − 126·08° (K salt) |
| Benzyl*iso*propylketone | 43, as benzyl*iso*propyl carbinol | − 54·9° (K salt) |
| Butyrophenone | 41, as benzyl-*n*-propyl carbinol | − 108·7° (Cd salt) |

**Substituted Acetophenones.** These ketones behave in a similar manner to acetophenone in that they are partly oxidised to aromatic acids and partly reduced to the corresponding secondary alcohols, but in addition certain other changes may appear. In the case of **o-aminoacetophenone**, no new reaction appears, for in the rabbit it forms *o*-aminophenylmethylcarbinol glucuronide and small amounts of anthranilic acid (Inagaki, 1933).

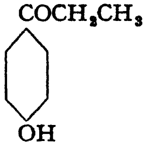


In this case the main reaction appears to be the reduction of the carbonyl group.

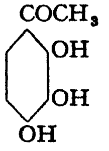
With *o*-**nitroacetophenone** little or no reduction of the carbonyl group takes place. The ketone is excreted partly unchanged and partly oxidised to *o*-nitrobenzoic acid. A certain proportion, however, is converted to indoxyl, which is excreted as indican (Böhm, 1938, see p. 225).



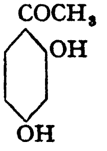
**Phenolic Ketones.** The introduction of hydroxyl groups into the aromatic ring hinders both the oxidation and the reduction of the carbonyl group. These ketones are excreted either unchanged or as glucuronides and ethereal sulphates. *p*-**Hydroxypropiophenone, gallacetophenone** and **resacetophenone** are excreted as conjugated sulphates and glucuronides both by dogs and rabbits, the keto group being unchanged (Nencki, 1894).



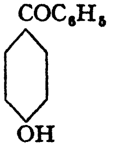
*p*-hydroxypropiophenone



gallacetophenone



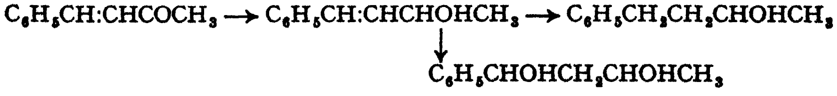
resacetophenone



*p*-hydroxybenzophenone

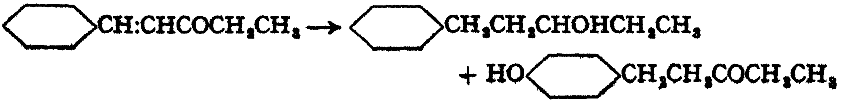
In the case of gallacetophenone, for example, Nencki searched for its possible oxidation product, pyrogallolcarboxylic acid, in the urine of dogs without success. *p*-**Hydroxybenzophenone** is said to be excreted unchanged, and does not increase the ethereal sulphate output (Schubenko, 1893).

**Unsaturated Ketones.** With unsaturated mixed ketones the changes are complex (Fischer and Bielig, 1940), and although both the unsaturated link and the keto group can be reduced *in vivo*, this does not happen invariably but depends both on the position of the keto group in the side-chain and on the nature of the alkyl group. In the case of **styrylmethyl ketone**, the unsaturated link is hydrogenated and the keto group asymmetrically reduced, the main metabolite being phenylethyl methyl carbinol. Styrylmethyl carbinol, which is probably the precursor of phenylethyl methyl carbinol, is also obtained in small amounts and traces of a diol, probably 4-phenylbutane-2:4-diol, are also eliminated.



$$\begin{tikzcd} C_6H_5CH{:}CHCOCH_3 \arrow[r] & C_6H_5CH{:}CHCHOHCH_3 \arrow[r] \arrow[d] & C_6H_5CH_2CH_2CHOHCH_3 \\ & C_6H_5CHOHCH_2CHOHCH_3 & \end{tikzcd}$$

The main metabolite of **styrylethyl ketone** is phenylethyl ethyl carbinol. In addition to this, a small amount of *p*-hydroxyphenylethyl ethyl ketone is formed, indicating that the ring has suffered oxidation and, although the double link has been hydrogenated, the keto group is unchanged.



Reduction of the double bond apparently does not take place when the keto group is attached directly to the ring, for if **phenylpropenyl ketone** is administered the only metabolite that can be isolated is phenylpropenyl carbinol.

$$C_6H_5COCH{:}CHCH_3 \rightarrow C_6H_5CHOHCH{:}CHCH_3$$

With ketones possessing two double bonds in the side-chain, as in the case of **6-phenyl-2-keto-$\Delta^{3:5}$-hexadiene**, only one is hydrogenated. This ketone is converted to a number of compounds in the rabbit ; the double bond $\alpha\beta$ to the $>$CO group is hydrogenated, but the other double bond appears in all the metabolites excreted. The main products are 6-phenyl-$\Delta^5$-hexene-2-ol and a dihydroxyphenylhexene.

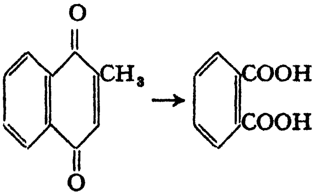
$$C_6H_5CH{:}CH.CH{:}CHCOCH_3 \rightarrow C_6H_5CH{:}CHCH_2CH_2CHOHCH_3$$

## QUINONES

***p*-Benzoquinone.** This substance is reduced in the dog and the rabbit to hydroquinone and the product excreted conjugated with sulphuric acid and probably glucuronic acid (Cohn, 1893*a*). Its trichloro- and tetrachloro-derivatives behave similarly (Schulz, 1892).

**2-Methyl-1:4-napthaquinone.** This substance is sometimes known as vitamin $K_3$ because it brings about the same biological response as the natural vitamins $K_1$ and $K_2$. When injected as its bisulphite derivative into either man or dogs it is converted into phthalic acid which can be isolated from the urine (Shemiakin and Schukina, 1944).

The antihaemorrhagic action of quinones of the vitamin K type only appears some time after administration and it is probable that they are first converted into a metabolite which is the actual carrier of their antihaemorrhagic functions. Phthalic acid (see p. 122) and some of its derivatives possess antihaemorrhagic activity and the formation of phthalic acid from vitamin $K_3$ may explain the activity of the latter (cf. dicoumarol, p. 227). This point is as yet controversial.



## ACID AMIDES AND IMIDES

Aromatic acid amides are hydrolysed *in vivo* to the corresponding aromatic acids which are then excreted combined usually with glycine. The general reaction can be represented as follows :—

$$ArCONH_2 \rightarrow ArCOOH \rightarrow ArCONHCH_2COOH$$

In the aliphatic series some amides undergo hydrolysis whereas others, such as biuret, are excreted unchanged. Gonnermann (1902) has shown that the liver and kidney tissues of the sheep contain enzymes which hydrolyse both aliphatic and aromatic acid amides.

**Formamide,** $HCONH_2$, is excreted by dogs mainly as formic acid (Halsey, 1898) and Gonnermann (1902) has shown that it is hydrolysed by both liver and kidney brei. **Benzylidenediformamide,** $(HCONH)_2CHC_6H_5$, is to a small extent excreted by the dog as hippuric acid (Bülow, 1894). The metabolism of **acetamide,** $CH_3CONH_2$, has received the attention of several workers. In the dog it is excreted unchanged, but in the rabbit it is partly hydrolysed to acetic acid and partly excreted unchanged (Schultzen and Nencki, 1872; Salkowski, 1877; Rudzki, 1876; Völtz and Yakusa, 1908). It is also hydrolysed by sheep-liver brei but not by kidney (Gonnermann, 1902). **Benzylidenediacetamide,** $(CH_3CONH)_2CHC_6H_5$, is excreted unchanged by the dog (Bülow, 1894).

**Oxamide,** $NH_2COCONH_2$, is partly converted to oxalic acid and partly excreted unchanged (Kobert and Küssner, 1879; Ebstein and Nicolaier, 1897). It is not hydrolysed by liver or kidney brei. **Biuret,** $NH_2CONHCONH_2$, and **oxamic acid,** $COOHCONH_2$, are excreted unchanged by the dog (Schwarz, 1898*a*; Koehne, 1894).

**Benzamide,** $C_6H_5CONH_2$, is converted by dogs and rabbits into benzoic acid which is excreted mainly as hippuric acid (Nencki, 1873; Bray, Neale and Thorpe, 1946). Baumann and Herter (1877), however, found that it increased the output of ethereal sulphates in dogs and this suggests that some benzamide is oxidised to a phenolic compound. The ethereal sulphate output is not increased in rabbits (Bray *et al.*, 1946).

Many amides including benzamide possess some narcotic activity (Nebelthau, 1895) which is not shown by the corresponding acids to which they are metabolised.

In 1890 Cohn made the interesting observation that if dogs were given large doses of benzaldehyde, he could isolate from their urine small amounts of benzamide. With rabbits, however, no benzamide was formed. According to Cohn the formation of benzamide from benzaldehyde takes place *via* ammonium benzoate thus:—

$$C_6H_5CHO \xrightarrow{+O} C_6H_5COOH \xrightarrow{+NH_3} C_6H_5COONH_4 \xrightarrow{-H_2O} C_6H_5CONH_2$$

Although Cohn's work has not been substantiated by other workers, some support for his claim is forthcoming from the discovery in animal tissues of an enzyme, benzamidase, which converts benzoic acid into benzamide. Waelsch and Busztin (1937) have found that horse-kidney brei or a glycerol extract of horse kidney, but not dry kidney powder (cf. glutaminase), shows benzamidase activity. The enzyme catalyses the reversible reaction:—

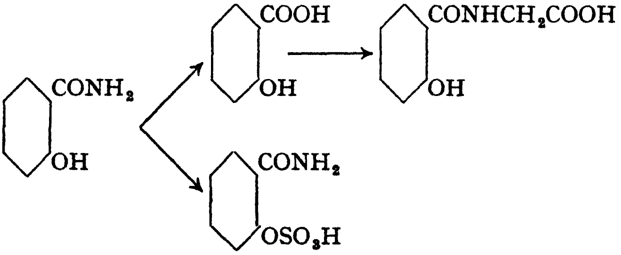
$$C_6H_5COOH + NH_3 \rightleftharpoons C_6H_5CONH_2 + H_2O$$

It has an optimum *p*H of 7·3 and works best when the benzoic acid concentration is 0·002 molar; higher concentrations of benzoic acid inhibit the formation of benzamide. It is present in liver, kidney and blood, but not in muscle, suprarenal and intestinal tissue. Its activity in the body, however, is probably limited for it is inhibited by glycine and it has to compete with hippuricase for benzoic acid.

**Dibenzamide,** $(C_6H_5CO)_2NH$, and **benzoylurea,** $C_6H_5CONHCONH_2$, when fed to dogs appear in the urine mainly as hippuric acid (Koehne, 1894).

**Phenylacetamide,** $C_6H_5CH_2CONH_2$, is largely transformed in the rabbit to phenaceturic acid (Bray *et al.*, 1946).

**Salicylamide** is excreted by dogs and rabbits as an ethereal sulphate (Baumann and Herter, 1877; Baas, 1889; Williams, 1938). It is also partly excreted as salicyluric acid (Baas, 1889). Conjugation with sulphuric acid probably precedes hydrolysis to salicylic acid, for the latter when fed is not excreted as an ethereal sulphate:



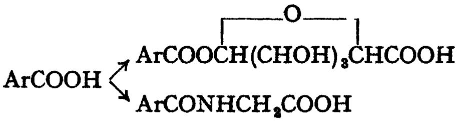
**Imides,** or the cyclic secondary amides of dicarboxylic acids, as far as is known, are converted into the corresponding dicarboxylic acids *in vivo.* **Succinimide** is completely metabolised in the dog, probably forming succinic acid. **Phthalimide** causes a rise in ethereal sulphates in the dog but otherwise its metabolic fate is obscure (Koehne, 1894).

## *Chapter Seven*

# The Metabolism of Aromatic Acids

THE aromatic acids are unique in that they are practically the only compounds known which are excreted by the animal body combined with glycine. The synthesis of hippuric acid from benzoic acid and glycine was one of the earliest of the synthetic mechanisms of the animal body to be recognised, and since its discovery numerous other aromatic acids have been found to undergo similar syntheses *in vivo.*

In general, the aromatic carboxyl group may be excreted combined with glycine as a hippuric acid (aroylglycine) and with glucuronic acid as an ester glucuronide in all species except the fowl.



$$\mathrm{ArCOOH}\;\begin{array}{l} \nearrow \mathrm{ArCOO\overbrace{CH(CHOH)_3CH}^{O}COOH} \\ \searrow \mathrm{ArCONHCH_2COOH} \end{array}$$

The quantities of these two main metabolic products formed and excreted, however, are dependent to a large extent upon the chemical nature of other substituents which may be present in addition to the carboxyl group. Furthermore, the metabolites excreted may vary from one species to another, particularly in quantity and sometimes even in nature. In some species it has been found that specific aromatic acids may be eliminated combined with the amino acids, glutamine and ornithine, but these mechanisms are unusual inasmuch as the glutamine mechanism is only found in man and the higher apes where it is limited to phenylacetic acid, whereas the ornithine synthesis is probably confined to birds. Variable quantities of aromatic acids may also be excreted uncombined and here the quantity of unchanged acid excreted depends mainly on the nature of any other substituent which may be present in the aromatic ring.

In some cases the aromatic nucleus may suffer oxidation *in vivo* with the production of small amounts of phenolic acids.

### BENZOIC ACID

The fate of benzoic acid in the animal body has received much attention, for, since the discovery of the hippuric acid synthesis over a century ago, benzoic acid has been employed in numerous studies on the biological synthesis and utilisation of the amino acid glycine.

Two metabolites of benzoic acid are known, namely, hippuric acid and benzoylglucuronic acid. There is no evidence to show that benzoic acid is oxidised to a phenolic acid. Herbivorous animals appear to excrete benzoic acid almost entirely as hippuric acid, whereas carnivores may in some circumstances excrete almost as much benzoylglucuronic as hippuric acid. Gupta (1932) stated that Indian cattle excrete administered benzoic acid entirely as hippuric acid, and Ringer (1911) showed that a goat could excrete a 25 g. dose entirely as the glycine conjugate. In the sheep benzoic acid is excreted almost completely as hippuric acid unless very large doses are fed, in which case benzoylglucuronic acid is also observed in the urine (Magnus-Levy, 1907). In this respect the omnivorous animals probably take an intermediate position between the herbivores and carnivores. Man (Magnus-Levy, 1907 ; Dakin, 1909–10) and the pig (Czonka, 1924) excrete appreciable amounts of benzoylglucuronic acid when given relatively large doses of benzoic acid, but man at least can eliminate small doses of benzoic acid entirely as hippuric acid. The dog appears to be the only carnivorous animal studied and according to Quick (1931), the production of hippuric acid in response to ingested benzoic acid is a relatively small and comparatively fixed amount for glucuronic acid plays a more important rôle than glycine. Hippuric acid is also formed by the frog (amphibian) and the turtle (aquatic reptile) (Komori, Sendju, Sagara and Takamatzu, 1926).

The question of the source of glycine in hippuric acid synthesis has interested many investigators, and there is consequently a fairly extensive literature on the subject. The evidence accumulated shows that glycine for hippuric acid synthesis can be derived from endogenous glycine which may be formed from the protein material of the body. The extent to which glycine can be synthesised in the body, however, is limited. In most animals preformed glycine present in the diet can be synthesised into hippuric acid. The preformed glycine may be present as such or in a protein such as gelatin.

When limited doses of benzoic acid are fed, hippuric acid can be formed and excreted without altering the level of the total nitrogen excreted and the nitrogen eliminated as hippuric acid appears in the urine at the expense of some of the urea nitrogen. Thus in a pig which had been reduced to a condition of minimal nitrogen metabolism by being kept on a carbohydrate diet, McCollum and Hoagland (1913) were able to show that if the dose of benzoic acid did not exceed 0·2 g./kg. no change occurs in the total nitrogen output and therefore in the protein catabolism; moreover, under these circumstances there was no change in the amounts of the nitrogenous components of the urine except in the case of urea which could be reduced from 50% of the total nitrogenous components to 19%. Similar results were obtained in human beings by Shiple and Sherwin (1922), who showed that doses of 3–10 g. benzoic acid eaten by a man who had been practically reduced to a state of endogenous protein metabolism caused no alteration in the total nitrogen excreted. When, however, relatively large doses are fed an enhanced excretion of urinary nitrogen does take place in most animals (e.g. Magnus-Levy, 1907, sheep and rabbits ; Salkowski, 1877–8, dogs ; Ringer, 1911, goats ; Czonka, 1924, pigs).

That the nature of the protein of the diet influences hippuric acid excretion has been demonstrated by several workers (e.g. Griffith and

Lewis, 1923). Czonka (1924) showed that in the pig, the nature of the protein in the diet influences the excretion of both hippuric acid and benzoylglucuronic acid. In the pig some 80–90% of ingested benzoic acid (dose 1 g./kg.) can be accounted for as the hippuric, benzoylglucuronic and free benzoic acids excreted in the urine. About 1–2% of the dose is always excreted as free benzoic acid. The relative amounts of the two conjugated metabolites are related to the quantity of preformed glycine in the diet, although the total amount of conjugated benzoic acid eliminated is not dependent on the nature of the diet since about 98% of the benzoic acid excreted is always in a combined form. On a protein-free diet the pig excretes 60% of the combined benzoic acid as hippuric acid and the rest as benzoylglucuronic acid. If casein (which contains no glycine) is added to the diet the output of hippuric acid rises very slightly to 63%. Beef meat and beef powder which are both low in glycine behave in a similar manner to casein. But addition of gelatin to the diet raises the hippuric acid output to as much as 89% of the total combined benzoic acid excreted, whilst the output of benzoylglucuronic acid decreases to 10%. In fact, gelatin is more effective than free glycine, which raises the hippuric acid output to 75–80%. Czonka's work shows that to a limited extent, there is a direct relationship between the glycine content of the protein fed and the amount of hippuric acid excreted; the relationship is of course inverse for benzoylglucuronic acid.

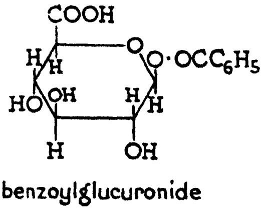
The above relationship between hippuric acid and preformed glycine is not applicable to the dog where the acid fed is benzoic acid. In this animal the glucuronic acid conjugate is excreted in greater amount than hippuric acid unless very small doses are fed, for with doses of 0·4–0·5 g./kg. twice as much benzoylglucuronic acid is excreted as hippuric acid. Furthermore, gelatin and glycine are not very effective in raising the hippuric acid output. In fact, the dog appears to possess only a limited capacity to produce glycine and to utilise preformed glycine for the formation, in particular, of hippuric acid in response to ingested benzoic acid (Quick, 1931*a*), but when phenylacetic acid is ingested the dog can utilise preformed glycine as well as any other animal for the synthesis of phenaceturic acid (Quick, 1932). Quick (1932; 1932*a*) has attempted to explain this peculiar phenomenon in the dog. The dog is peculiar in that hippuric acid synthesis only takes place in the kidney, whereas in other animals the synthesis can also take place elsewhere in the body. Friedmann and Tachau (1911), for example, have shown that nephrectomised rabbits can synthesise hippuric acid, whereas nephrectomised dogs cannot. According to Quick, absorbed benzoic acid is probably first converted to benzoylglucuronic acid in the liver. This acid then passes into the blood stream, which probably contains very little free benzoic acid, to the kidney. In the latter organ, some of the glucuronide, dependent on its rate of excretion, is hydrolysed and the benzoic acid set free combines with glycine. The chances are, therefore, that more of the glucuronide will be excreted than glycine conjugate. In other animals both the hippuric acid and the glucuronide are formed simultaneously, and hence the ratio of hippuric acid to glucuronide would tend to be greater than in the dog. In the case of phenylacetic acid, the dog excretes more glycine conjugate than glucuronide for here the synthesis of phenaceturic acid is not confined to the kidney.

It has been mentioned above that in most animals benzoylglucuronic acid only appears in the urine when rather large doses of benzoic acid are fed. This fact may indicate that there is a temporary exhaustion of the glycine supply for the detoxication of large doses of benzoic acid and the glucuronic acid mechanism comes into play as a second defence mechanism. There is, however, some uncertainty as to whether this is a correct interpretation, for Czonka has shown that in the pig benzoylglucuronic acid is formed at a greater rate in the early hours after benzoic acid ingestion than in later ones. However, Czonka has interpreted this observation as meaning that the mobilisation of glycine is probably slow and that the absorption of benzoic acid is faster than the mobilisation of glycine for hippuric acid synthesis, and glucuronide formation therefore becomes a substitute detoxication process for any benzoic acid not yet combined with glycine.

The rate of mobilisation of glycine for benzoic acid detoxication apparently varies from animal to animal and Quick (1931*b*) has calculated, very roughly, this rate for a number of animals; in the dog the rate is 3·5 mg./hr.; man, 9·0; the pig, 15·0; the rabbit, 24·0.

The relationship between preformed glycine and hippuric acid excretion has also been investigated by the use of isotopes, but as yet the results are not fully consistent. Schoenheimer, Rittenberg, Fox, Keston and Ratner (1937) first reported that, using both isotopic hydrogen and nitrogen as markers, about 75% of hippuric acid administered to rats is excreted in the urine without undergoing hydrolysis and that benzoic acid combines directly *in vivo* with dietary glycine. Then Rittenberg and Schoenheimer (1939), using glycine containing $N^{15}$, found that only about one-third of the isotopic glycine fed with benzoic acid was excreted as hippuric acid, the greater proportion of the hippuric acid glycine being derived from endogenous sources. But Waelsch and Rittenberg (1939) find as much as 55% of the hippuric acid glycine to be derived from dietary isotopic glycine. Further work will, no doubt, elucidate these inconsistencies.

Benzoylglucuronic acid is an interesting compound from the purely chemical point of view and although there has been some controversy regarding its structure, its molecular constitution is now definitely established. It was first isolated from the urine of sheep receiving large dozes of benzoic acid by Magnus-Levy (1907), who obtained it as a strychnine salt and as an amorphous dextrorotatory sodium salt. Magnus-Levy assigned to it the structure of a 1-benzoylglucuronic acid, although he adduced no experimental evidence in support. In 1926 Quick isolated a crystalline laevorotatory benzoylglucuronic acid from the urine of dogs fed benzoic acid. This compound undergoes a rapid change in rotation in the presence of alkali which Quick claimed to be mutarotation. It also reduces the common sugar reagents and appears to combine with sodium cyanide. These facts were interpreted by Quick as meaning that the aldehydic group of the glucuronic acid residue did not carry the benzoyl group and was in fact free, thereby exhibiting mutarotation, reduction and cyanohydrin formation. Quick therefore postulated that the benzoyl radicle was attached to a carbon atom of the glucuronic acid residue other than the first and favoured a 2-benzoylglucuronic acid structure. Pryde and Williams (1933) pointed out that the properties of benzoylglucuronic acid were consistent with the 1-benzoyl structure of Magnus-Levy, for the observations of Quick could be explained by the labile nature, in the presence of alkali, of the ester linkage joining the benzoyl radicle to the glycosidic carbon atom of glucuronic acid. The problem was finally settled by Goebel (1938), who demonstrated that 1-benzoyl-2:3:4-triacetylglucuronide methyl ester prepared by synthesis was identical with that prepared from natural benzoylglucuronic acid. The structure of the acid was therefore established beyond doubt as being 1-benzoylglucuronide. The changes in specific optical rotation in the presence of alkali could not therefore be due to mutarotation as postulated by Quick, and Goebel suggests that these changes may be due to a complex series of reactions initiated by a migration of the benzoyl group, catalysed by hydroxyl ions, from the aldehydic to one of the remaining carbon atoms of the uronic acid. The reducing power of the acid is no doubt due to removal of the benzoyl group by the alkalinity of the reagents used, thereby releasing a reducing group. Whereas the benzoylglucuronic acid prepared by Quick was laevorotatory, the amorphous sodium salt prepared by Magnus-Levy was dextrorotatory. According to Goebel the latter salt is probably a rearranged form of laevorotatory benzoylglucuronide, for it was isolated from an alkaline urine which had been standing for some time.

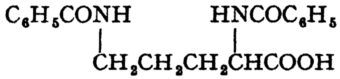


benzoylglucuronide

A number of ester glucuronides of the benzoylglucuronide type have been isolated from urine, and these are listed in Table 14.

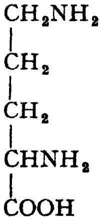
### The Fate of Benzoic Acid in the Hen

The formation of hippuric acid in response to ingested benzoic acid does not occur in the hen, and it would appear that birds characteristically excrete benzoic acid combined with the amino acid ornithine. The ormation of ornithuric acid may be common to all birds, but this has not yet been tested experimentally. The formation of ornithuric acid was first discovered by Jaffe (1877), who was searching for hippuric acid in the excreta of hens receiving benzoic acid. He failed to find hippuric acid but isolated a compound having the constitution of a $\alpha$:$\delta$-N:N'-dibenzoylornithine.



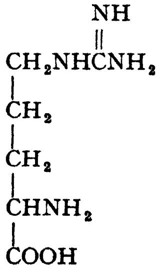
$$\begin{array}{l} C_6H_5CONH \qquad HNCOC_6H_5 \\ \;|\qquad\qquad\qquad\quad | \\ CH_2CH_2CH_2CHCOOH \end{array}$$

Apparently the hen cannot utilise glycine for the detoxication of benzoic acid even when fed an excess of glycine (Yoshikawa, 1910). Simultaneous feeding of benzoic acid and glycine results in the excretion of ornithuric acid without a trace of hippuric acid. Ornithuric acid is formed in the hen, according to Crowdle and Sherwin (1923*a*), at the expense of waste nitrogen, i.e. uric acid. Crowdle and Sherwin (1923*a*) provided hens with artificial ani whereby the urine could be collected separately from the faeces. By this means they were able to show that on giving hens benzoic acid there was a drop in the uric acid nitrogen (which normally constitutes some 70–80% of the total urinary nitrogen of fowls), but the total nitrogen was unchanged. The drop in the uric acid nitrogen could be accounted for by the ornithine excreted combined with benzoic acid. Feeding ornithine increases the ornithuric acid output, but other amino acids have little effect. Ornithine is closely related chemically and biologically to the amino acid, arginine. Both these acids, according to Krebs and Henseleit (1932), play an important part in the synthesis of urea in the mammalian organism.



$$\begin{array}{l} CH_2NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

ornithine



$$\begin{array}{l} \qquad\qquad NH \\ \qquad\qquad \| \\ CH_2NHCNH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

arginine

The synthesis of ornithuric acid in the hen naturally raises the question of the importance of ornithine and arginine in avian metabolism. Arginine is not an essential amino acid for mammals but in the chick it appears to be a growth factor (Arnold, Kline, Elvehjem and Hart, 1936; Klose, Stokstad and Almquist, 1938). In chicks ornithine cannot replace arginine as a growth factor and the chick obviously lacks the mechanism for arginine formation and decomposition that exists in mammals. The formation of urea in mammals through the mechanism of the Krebs cycle is associated with the occurrence of the enzyme arginase in the liver. Arginase is not found in the liver of the hen, which excretes less than

1% of its waste nitrogen as urea, but it is present in small amounts in its kidney (Hunter and Dauphinee, 1925). It has been pointed out by Klose *et al.* (1938) that this small amount of arginase in the kidney of the fowl may be connected with the ornithuric acid synthesis. Crowdle and Sherwin, as mentioned above, reported that the hen could synthesise ornithine for conjugation with benzoic acid on a diet low in arginine. The figures for the nitrogen partition in hen urine given in the paper of Crowdle and Sherwin show that when benzoic acid is administered there is a rise in the urea output which is between five and twenty times that of the urea excretion before benzoic acid feeding. This extra urea production, coupled with the occurrence of arginase in the kidney, may indicate that the ornithine for conjugation was actually derived from the arginine of the tissues or from the diet (polished rice, potatoes and yeast) which undoubtedly contained a small amount of arginine. Obviously further work is necessary on the relationship between the ornithuric acid synthesis and avian arginine requirements.

Although glycine is not an essential amino acid for most mammals, in the hen it is, like arginine, a growth factor (Almquist, Stokstad, Mecchi and Manning, 1940 ; Hegsted, Briggs, Elvehjem and Hart, 1941). In addition to glycine another mammalian detoxicating agent, namely, glucuronic acid, has been found essential for growth in chicks. Almquist and Mecchi (1940) found that polished rice contained growth factors for chicks. These were identified as glycine and chondroitin and further examination of the growth-promoting properties of chondroitin revealed that the essential factor in chondroitin was the glucuronic acid and not the galactosamine part of the molecule. Whether the hen uses glucuronic acid as a detoxicating agent is apparently not known.

## THE METABOLISM OF SUBSTITUTED BENZOIC ACIDS

The metabolism of substituted benzoic acids in the animal body is qualitatively similar to that of benzoic acid and the majority of these acids form glycine and glucuronic acid conjugates. The relative amounts of these conjugates as well as of the unchanged acids are dependent on the nature and position of the substituents. *Meta* and *para* substituents have very little influence on the extent to which the carboxyl group conjugates with glycine except when the substituent is OH, and in this case the output of substituted hippuric acid is diminished. When the aromatic acid is *ortho* substituted, one finds that the glycine conjugation is considerably diminished irrespective of the nature of the group. *Ortho* substitution of an aromatic acid, therefore tends to inhibit the conjugation of the aromatic carboxyl group with glycine and the inhibition is independent of the type of group present. In the glucuronic acid conjugation, the nature of the substituent group is of great importance, since acidic groups in the *ortho* and *para* position tend to diminish the output of ester-glucuronide, whereas basic groups tend to increase the output. Neutral groups in these positions have little influence. *Meta* substitution has little effect on the glucuronic acid output and what little effect it does have tends in general to be opposite to that of the *ortho* substituents. The above generalisations were found by Quick (1932) to operate in the organism of the dog, and as yet there is no evidence that they are applicable to other animals.

The following monosubstituted aromatic acids have been shown to form hippuric acids and/or ester glucuronides in various animals:— *o*-, *m*-, *p*-toluic acids; *o*-, *m*-, *p*-chlorobenzoic acids; *o*-, *m*-, *p*-bromobenzoic acids; *o*-, *m*-, *p*-iodobenzoic acids; *o*-, *m*-, *p*-fluorobenzoic acids; *o*-, *m*-, *p*-hydroxybenzoic acids; *o*-, *m*-, *p*-aminobenzoic acids; *o*-, *m*-, *p*-methoxybenzoic acids; *m*-, *p*-nitrobenzoic acids (Quick, 1932, 1932*b*; Novello, Miriam and Sherwin, 1926; Muenzen, Cerecedo and Sherwin, 1926) (see Table 15). It is not proposed to give an account of all these acids separately; only those which are of special importance in biochemistry will be discussed.

## THE HYDROXYBENZOIC ACIDS

**Salicylic Acid** (*o-hydroxybenzoic acid*). Salicylic acid and its derivatives have been extensively used in medicine for the last half-century or more. To mention only two of their uses, salicylates are widely used in the treatment of rheumatic conditions, and the well-known drug aspirin or acetylsalicylic acid is used as an analgesic and antipyretic. There is consequently an extensive literature on the metabolism and excretion of salicylates.

As a result of considerable work it is now fairly clear what metabolic products are excreted after the ingestion of salicylates. The urine may contain free salicylic acid, salicyluric acid, gentisic acid, uraminosalicylic acid (Baldoni's "*uraminosalicylsäure*"), salicylglucuronic acid and salicyl ethereal sulphates. The constitutions of the latter three compounds are unknown. All these compounds may not be excreted by a single species, but all are reported as occurring in the urine of one species or another. The human being, for example, is said to excrete all the above metabolites except the ethereal sulphates.

Studies of the fate of salicylic acid in the organism have yielded many conflicting results, but recent work, particularly by Quick (1933) and by Kapp and Coburn (1942), has given a fairly clear picture of the metabolism of salicylic acid in the human being. Compared with benzoic acid, salicylic acid is only slowly eliminated, for whilst a 2 g. dose of benzoic acid is excreted in about 4 hours, only about 50% of a similar dose of salicylic acid is eliminated in 24 hours (Quick). Kapp and Coburn found about 60% of a 1·25 g. dose to be eliminated in 24 hours. Some 80% of ingested salicylic acid can be accounted for in the urine as compounds containing the salicyl radicle, whilst some 4–8% is excreted as dihydroxy acids (Kapp and Coburn). Of the total salicyl excreted, some 20% appears as free salicylic acid (Kapp and Coburn, 1942; Quick, 1933; Holmes, 1925). The main product of salicylic acid metabolism in man is salicyluric acid (*o*-hydroxyhippuric acid, m.p. 168–169°). It was first isolated by Bertagnini in 1856 from urine after salicylic acid ingestion, and he named it salicyluric acid by analogy with hippuric acid. About 50–60% of the total salicyl radicle excreted is present as this glycine conjugate (Kapp and Coburn; Quick; Holmes).

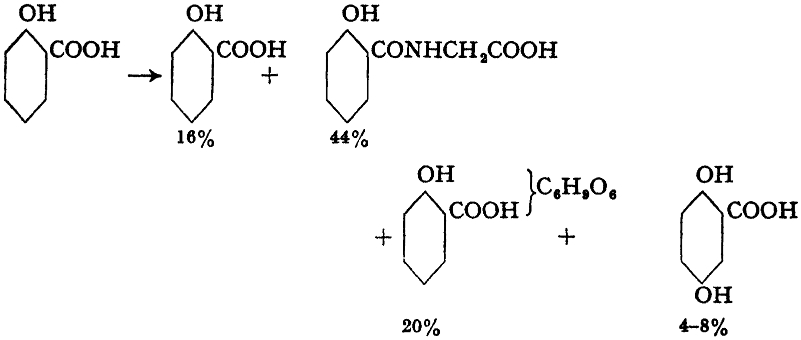
In man, salicylic acid does not increase the ethereal sulphate output (Baumann and Herter, 1877), and presumably its phenolic hydroxyl is so placed in relation to the carboxyl group that conjugation with sulphuric acid is hindered.

There is little doubt that salicylic acid is excreted combined with glucuronic acid, and Kapp and Coburn estimate that man excretes about 25% of the total salicyl accounted for, as salicylglucuronides. The possible occurrence of salicylglucuronides was first reported by Baldoni (1906), and their occurrence in dog " salicylic acid " urine was reported by Neuberg (1911) and by Quick (1932, 1932*b*). But so far no one has succeeded in isolating these compounds. Kapp and Coburn, working with human urine, have obtained evidence indicating that the glucuronides may have both the ester and ether glucuronide type of linkages (cf. *p*-hydroxybenzoic acid). According to Lutwak-Mann (1942) the intact rat is unable to synthesise salicylglucuronides and, furthermore, rat-liver slices which can synthesise conjugated glucuronic acid in the presence of benzoate, *m*- and *p*-hydroxybenzoate and *o*- and *p*-aminobenzoate, are unable to perform this synthesis when salicylate is present.

Ingested salicylic acid undergoes oxidation in the human being to gentisic acid (3:5-dihydroxybenzoic acid, m.p. 198–200°) (Baldoni, 1908; Angelico, 1921) to the extent of some 4–8% (Kapp and Coburn, 1942). Gentisic acid is also excreted in the urine of rats receiving sodium salicylate, methyl salicylate and sodium acetylsalicylate, but not by rats receiving salol (phenyl salicylate) and sodium sulphosalicylate (Lutwak-Mann, 1943). A compound closely related to gentisic acid was isolated from dog urine by Baldoni (1908) who named it " uraminosalicylsäure ", but left its structure undetermined. A similar compound (m.p. 169–170°) has also been isolated in very minute amounts from human urine by Kapp and Coburn who, from a study of its titration curve and other properties, came to the conclusion that Baldoni's " uraminosalicylsäure " might be a compound of glycine, salicylic acid and gentisic acid, and they suggested for it the formula, $HOC_6H_4.N(CH_2COOH).C_6H_2(OH)_2COOH$. The possibility that it might be an artefact has not been mentioned.

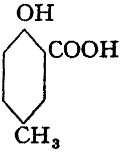
In patients suffering from acute rheumatism less salicyluric acid (40%) is excreted than in normals, whilst the gentisic acid output is two to four times greater than normal. The output of salicylglucuronides, however, appears to be unaltered in the rheumatic patient (Kapp and Coburn).

In the human being the fate of salicylic acid can be represented as follows :—



Salicylic acid is said to be excreted as an ethereal sulphate in the dog (Baumann and Herter, 1877) but not in the rabbit (Williams, 1938). Both salicyluric acid and salicylglucuronide are excreted by the dog, but the latter in greater quantities than the former (Quick, 1932). Gentisic acid has also been isolated from dog urine by Neuberg (1911) after the administration of lithium aspirin. There is little doubt that the metabolism of salicylic acid in the dog is qualitatively similar to that in the human being, any differences being in the quantitative relationships of the metabolites.

The homologues of salicylic acid, the *o*-, *m*- and ***p*-cresotic acids** have both antiseptic and antipyretic properties. The fate of ***p*-cresotic acid** (*p*-homosalicylic acid) in the dog has been investigated by Loesch (1932),

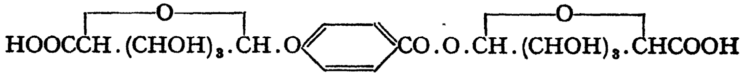


who found that, unlike salicylic acid, it did not form a glycine conjugate. In dogs it is excreted partly unchanged and partly as a glucuronide, but it does not form an ethereal sulphate.

***m*-Hydroxybenzoic Acid.** In man this acid is mainly excreted combined with glycine (*m*-hydroxyhippuric acid, m.p. 182–3°) and only small amounts are conjugated with glucuronic acid. Its metabolism closely resembles that of benzoic acid, although perhaps slightly more is excreted in the free state (Quick, 1932*b*). In the dog it forms not only a glycine and a glucuronic acid conjugate (Quick), but also an ethereal sulphate (Baumann and Herter, 1877). Some 4% of ingested *m*-hydroxybenzoic acid is excreted as an ethereal sulphate in the rabbit (Williams, 1938).

***p*-Hydroxybenzoic Acid.** In man, the main bulk of ingested *p*-hydroxybenzoic acid is excreted free and as *p*-hydroxyhippuric acid, the amount of glucuronide formed being extremely small (Quick, 1932*b* ; Schotten, 1882). In the rabbit some 6–8% is excreted as an ethereal sulphate (Williams, 1938).

Its metabolism in the dog is of special interest since it is mainly excreted as a compound containing two glucuronic acid residues (Quick, 1932*b*), one attached to the aromatic ring by an ether glycosidic linkage and the other by an ester glycosidic linkage. *p*-Glucuronosidobenzoylglucuronide (*p*-hydroxybenzoic acid diglucuronic acid) forms white needles (melting with decomposition slightly above 200°) showing a laevorotation. Like benzoylglucuronide it shows a change in rotation in the presence of alkali, and by virtue of its ester glucuronide linkage it reduces the common sugar reagents on heating. Its structure and the configuration of its sugar linkages have not been determined, but it is probable that its glycosidic linkages are both of the *β*-type.



When fed to man, this diglucuronide is metabolised and slowly eliminated both as *p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid (Quick, 1932*b*). The dog also eliminates *p*-hydroxybenzoic acid to a small extent as *p*-hydroxyhippuric acid (m.p. 227°).

In hens *p*-hydroxybenzoic acid does not form an ornithuric acid as does benzoic acid, and is mainly excreted unchanged (Crowdle and Sherwin, 1923*b*).

## THE AMINOBENZOIC ACIDS

**Anthranilic Acid** (*o*-aminobenzoic acid). In dogs and rabbits the main bulk of ingested anthranilic acid is excreted as a glucuronide (Mitsuba and Ichihara, 1927). In the dog some 72% of the acid may be excreted as a glucuronide within 12 hours (Quick, 1932). Small amounts of *o*-uraminobenzoic acid[1] (*o*-NH$_2$CONHC$_6$H$_4$COOH) are also found in the urine. It does not, however, form a glycine conjugate in man, rabbits or dogs (Muenzen, Cerecedo and Sherwin, 1926). Small amounts of anthranilic acid are oxidised in dogs and rabbits since both species

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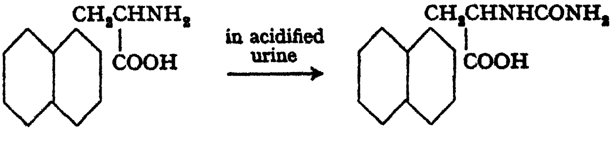
[1] A number of so-called "uramino" acids have been isolated from the urine of animals receiving amino acids. The isolation of these acids from urine must not be regarded as indicating that they are metabolites formed *in vivo*. Dakin (1922*d*) has pointed out that they are easily formed by the interaction, in urine, of amino acids with urea (see also Lippich, 1908). Thus the claim of Salkowski (1873) that taurine when fed to men or dogs is partly excreted as the substituted urea, NH$_2$CONHCH$_2$CH$_2$SO$_3$H, must be regarded as incorrect (Schmidt and Allen, 1920). ***dl*-α-Naphthylalanine** when fed to dogs or rabbits is partly excreted unchanged, but is isolated from the urine as *α*-naphthylalanine hydantoic acid which Friedmann (1931) proved to be an artefact:—



show an increased output of ethereal sulphates when fed with this acid (Mitsuba and Ichihara).

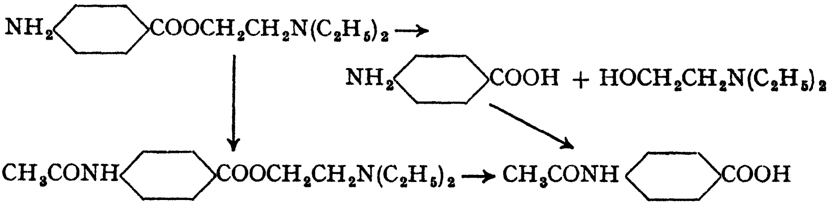
***m*-Aminobenzoic acid**, when fed to dogs is excreted combined with glucuronic acid to the extent of 50% of the ingested dose (Quick, 1932). It also forms small amounts of *m*-aminohippuric acid (m.p. 192°) in both dogs and rabbits (Salkowski, 1882).

***p*-Aminobenzoic acid.** *p*-Aminobenzoic acid has recently been recognised as a compound of considerable biological importance. It has been classified as a B vitamin and is an essential factor in the nutrition of certain micro-organisms (Fildes, 1940). It has the important property of inhibiting the action of sulphanilamide on bacteria (Woods and Fildes, 1940; Selbie, 1940) and is identical with the antisulphanilamide factor discovered by Woods (1940) in yeast extracts, from which it was isolated by Rubbo and Gillespie (1940). Deficiency of *p*-aminobenzoic acid in black or piebald rats causes the greying of the fur, and hence it has been called the chromotrichia factor for rats. In chicks it acts as a growth-promoting factor (Ansbacher, 1941) and appears to possess the essential properties of a vitamin (for further information see Ansbacher, 1944).

The inhibitory action of *p*-aminobenzoic acid on the bacteriostatic activity of the sulphonamides is most marked with sulphanilamide which it resembles most closely in chemical structure. Its effect on sulphapyridine is less and is still smaller on sulphathiazole (Strauss, Lowell and Finland, 1941). Modifications of the $-NH_2$ or $-COOH$ groups lower its antisulphanilamide activity, and Woods (1940) found that *p*-acetaminobenzoic acid had only one-half and *p*-aminobenzamide only two-thirds the activity of unaltered *p*-aminobenzoic acid. Since *p*-aminobenzoic acid undergoes modification of both its amino and carboxyl group in the body, its *in vivo* antisulphanilamide activity must be closely related to its metabolism (see McIlwain, 1940; 1943).

In the animal body it is known that *p*-aminobenzoic acid is excreted as a glucuronide and as an acetyl derivative. Earlier workers (Hildebrandt, 1903; Muenzen *et al.*, 1926) were unable to detect any conjugated products in the urine of men and dogs which had ingested this acid. However, Ellinger and Hensel (1914) showed that it was acetylated by the rabbit, and Harrow, Mazur and Sherwin (1933) showed that 25% of *p*-aminobenzoic acid injected subcutaneously into rabbits was excreted as *p*-acetaminobenzoic acid. *p*-Aminobenzoic acid is also acetylated in man, and Bernhard (1940) has shown that if deuteroacetic acid is fed simultaneously the excreted *p*-acetaminobenzoic acid contains deuteroacetyl to the extent of 9–12%. In man absorbed *p*-aminobenzoic acid is quickly excreted, and a dose of 4 g. is eliminated within 12 hours. About two-thirds of the ingested acid is eliminated in a form containing a free amino group, whereas the amino group of the other third is combined (Strauss, Lowell and Finland, 1941). According to Zehender (1943) 75–80% of a dose of 1–2 g. of *p*-aminobenzoic acid is excreted by man within 24 hours, and of this about two-thirds is acetylated for a 1 g. dose and nearly one-half for a 2 g. dose. *p*-Acetaminobenzoic acid is mainly excreted unchanged, for 63% of a 1 g. dose is excreted unchanged and no free aminobenzoic acid is formed. In dogs a considerable proportion (60–70%) of *p*-aminobenzoic acid forms a glucuronide, but this compound has not been isolated (Quick, 1932). There is no evidence to show that *p*-aminobenzoic acid forms *p*-aminohippuric acid *in vivo,* and Quick is of the opinion that the hippuric acid conjugate is not formed in the dog. *p*-Aminohippuric acid is of interest because it delays the excretion of administered penicillin (Beyer *et. al.,* 1944).

One of the most important derivatives of *p*-aminobenzoic acid is the local anaesthetic **diethylaminoethyl *p*-aminobenzoate** (novocaine, procaine). In dogs and rabbits it is probably rapidly broken down to *p*-aminobenzoic acid and diethylaminoethanol (Dunlop, 1935; Allen and Livingstone, 1942; McLachlin, 1945). In the rabbit it also undergoes conjugation which is probably acetylation of the aromatic amino group, but whether it is novocaine as such, or *p*-aminobenzoic acid produced from it, that is acetylated is not known. A possible scheme for the metabolism of novocaine is as follows:—



## PHENYLACETIC ACID AND ITS DERIVATIVES

The metabolism of **phenylacetic acid** has points of special interest since its metabolism in man and the chimpanzee involves a " detoxication mechanism " which is not used in other animals and which is specific for phenylacetic acid.

In the lower animals the metabolism of phenylacetic acid is qualitatively similar but quantitatively dissimilar to that of benzoic acid, and it is excreted as glycine and glucuronic acid conjugates. The fate of the acid in the dog was investigated by Quick (1932), who found that almost twice as much phenylacetic acid is excreted as phenaceturic acid than as phenylacetylglucuronide. The output of the glycine conjugate is markedly increased by feeding glycine. The synthesis of phenaceturic acid and of hippuric acid is carried out by two different enzymes, and this difference is related mainly to the nature of the side-chain. Benzoic acid and phenylacetic acid present two different stereochemical patterns for enzyme action. The importance of the side-chain as opposed to nuclear substituents is evident when the metabolisms of benzoic and *p*-chlorobenzoic acids are compared with those of phenylacetic and *p*-chlorophenylacetic acids, for there is fairly close agreement between the rate of excretion and the effect of exogenous glycine on the conjugation of benzoic and *p*-chlorobenzoic acids on the one hand, and a similar correspondence is found between phenylacetic and *p*-chlorophenylacetic acids on the other. The difference between benzoic acid and phenylacetic acid with reference to the effect of glycine on their metabolism has already been discussed (see p. 97).

Phenylacetic acid is excreted as phenaceturic acid (m.p. 142–143°) by the rabbit (Salkowski, 1877, 1882–3), the sheep (Vasiliu, 1909), and the horse (Salkowski, 1884, 1888). Phenaceturic acid occurs as a normal constituent of horse urine and was isolated from this source by Salkowski. It is also formed in the monkey, for Sherwin (1917) was able to isolate 0·82 g. (51% of dose) of phenaceturic acid from the urine of a female *Macacus rhesus* which had received 1 g. of phenylacetic acid. It is probable that substituted phenylacetic acids behave similarly in the lower animals, for Sherwin and Helfand (1919) found that ***p*-nitrophenylacetic acid** was excreted as *p*-nitrophenaceturic acid (m.p. 172–173°) to a small extent in the dog; they found 61·5% of a 12 g. dose of *p*-nitrophenylacetic acid to be excreted, 44% unchanged and 17% as *p*-nitrophenaceturic acid.

It has already been mentioned that the hen utilises ornithine instead of glycine for the conjugation of benzoic acid, and this is also true for phenylacetic acid which is excreted as phenacetornithuric acid (m.p. 139–139·5°) (diphenacetylornithine) (Totani, 1910).

$C_6H_5CH_2CONHCH_2COOH$

phenaceturic acid

$C_6H_5CH_2CONHCH_2CH_2CH_2CH(NHCOCH_2C_6H_5)COOH$

phenacetornithuric acid

$C_6H_5CH_2CONHCH(COOH)CH_2CH_2CONH_2$

phenacetylglutamine

Substituted phenylacetic acids are apparently excreted in the same way for Sherwin and Helfand have shown that when the fowl is given *p*-nitrophenylacetic acid a small amount is excreted free, but the main bulk forms *p*-nitrophenacetornithuric acid (di-(*p*-nitrophenacetyl)-ornithine, m.p. 184–185°), the barium, potassium and sodium salts of which are dextrorotatory.

In man glycine is not involved in the metabolism of phenylacetic acid and we find instead that glutamine plays the rôle of glycine. This interesting detoxication process was discovered by Thierfelder and Sherwin (1914, 1915), who isolated phenacetylglutamine (m.p. 109°, $[\alpha]_D$ — 18°) and its addition compound with urea, phenacetylglutamine urea, from urine of men receiving phenylacetic acid. These workers also discovered the remarkable fact that most substituted phenylacetic acids did not form glutamine conjugates in man, for ***o*-amino-, *p*-nitro-, *p*-amino-** and ***p*-hydroxyphenylacetic acids** pass through the body unchanged. In a careful experiment on man, Sherwin and Helfand were able to recover 68·7% of a dose of *p*-nitrophenylacetic acid unchanged from the urine and no other compound of this acid was found. In addition to phenacetylglutamine man excretes a small amount of phenacetylglucuronic acid, and after a single dose of phenylacetic acid 95% is excreted as the glutamine conjugate and 5% as the glucuronide. On continued ingestion of the acid there is a tendency for more of the glucuronide to be excreted (Ambrose, Power and Sherwin, 1933). The glutamine used in the conjugation process appears to be formed at the expense of waste nitrogen (see Table 10), for ingestion of phenylacetic acid leads to a decrease in the urea nitrogen output, but the total nitrogen is unaffected.

TABLE 10 (after Shiple and Sherwin, 1922)

*Formation of Glycine and Glutamine for Detoxication in Man at the Expense of Waste Nitrogen (Man on Low N Diet)*

| Day | Phenyl-acetic acid fed | Benzoic acid fed | Total N excreted | Urea N | Ammonia N | Glycine N | Glutamine N |
|---|---|---|---|---|---|---|---|
| 1 | — | — | — | — | — | — | — |
| 2 | — | — | 5·91 | 4 66 | 0·52 | — | — |
| 3 | — | — | 5·71 | 4·23 | 0·45 | — | — |
| 4 | — | 6·0 | 6·09 | 3·78 | 0·46 | 0·63 | — |
| 5 | 6·6 | — | 5·45 | 2·62 | 0·43 | — | 1·06 |
| 6 | 3·3 | 3·0 | 4·78 | 2·72 | 0·41 | 0·31 | 0·53 |
| 7 | 10·0 | — | 5·55 | 1·57 | 0·60 | — | 1·97 |

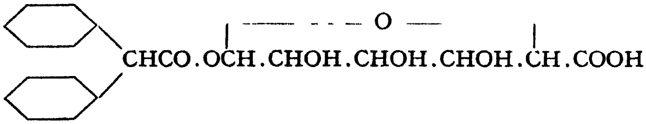
It has been mentioned above that in the *Macacus rhesus* monkey, phenylacetic acid forms a glycine conjugate, but in the anthropoid ape, its fate is similar to that in man. Power (1936) fed a young chimpanzee (" Buddy ", 2½ years old and weighing 10 kg.) at the New York Zoological Park with four doses of 1·5 g. of phenylacetic acid, the animal being maintained on a diet of milk, bananas and eggs. Phenacetylglutamine was excreted and accounted for 5 g. out of 6 g. of phenylacetic acid fed.

Phenylacetic acid has an interesting effect on the excretion of sulphur (Salkowski and Salkowski, 1879). In rabbits fed with this acid there is a large increase in the excretion of total sulphur with a concomitant decrease in the amount of sulphur excreted as ethereal sulphate. The decrease in the output of ethereal sulphate has been explained by the disinfecting action phenylacetic acid possesses on the intestine. Intestinal putrefaction is decreased, and thus the excretion of phenols and consequently of ethereal sulphate is lowered. This effect of phenylacetic acid on the ethereal sulphate output was confirmed by Ambrose *et al.* (1933) in man, but they observed no great increase in total sulphur excretion.

Some interesting experiments have been carried out on the feeding of the conjugated products of phenylacetic acid to animals in which they are not normally formed. Thus if phenylacetylglutamine is fed to dogs, cats or rabbits it is excreted unchanged and is not converted to phenaceturic acid ; it is also eliminated unchanged in the hen and no conversion to phenacetornithuric acid takes place. On the other hand, if phenaceturic acid and phenacetylornithine are fed to man they are *both* excreted unchanged and not as phenacetylglutamine. Similarly the ornithine conjugate when fed to dogs is eliminated unchanged (Sherwin, 1922). Apparently phenylacetylated amino acids of the type $RCH(NHCOCH_2C_6H_5)COOH$ are not metabolised by animals since not only the above naturally formed phenaylcetylated amino acids, but also synthetic compounds such as the N-phenylacetyl derivatives of alanine, leucine, asparagine, aspartic acid and glutamic acid are excreted unchanged (Sherwin, 1922; Shiple and Sherwin, 1922*a*).

The *o*-, *m*- and *p*-**aminophenylacetic acids** are acetylated in man and in the rabbit (Cerecedo and Sherwin, 1923–4, 1924–5; Muenzen, Cerecedo and Sherwin, 1926*a*).

**Diphenylacetic Acid.** Diphenylacetic acid does not behave in man in the same way as phenylacetic acid. Its metabolism is similar in man, the rabbit and the dog (Miriam, Wolf and Sherwin, 1927*a*), the only conjugate formed being diphenylacetylglucuronide (decomposes at 180–185°; $[\alpha]_D - 34{\cdot}75°$) :—



In man, the rabbit and the dog a glycine conjugate is not formed, although synthetic diphenylacetylglycine (m.p. 157°) has been prepared. In the rabbit some 50% of the acid can be recovered unchanged. The glucuronide is a typical ester glucuronide since it reduces the common sugar reagents on warming and becomes dextrorotatory in alkaline solutions. It forms no cyanohydrin and does not react with phenylhydrazine (Miriam, Wolf and Sherwin, 1927).

**Triphenylacetic acid,** $(C_6H_5)_3C.COOH$, is largely excreted unchanged in the dog and in the rabbit, for no conjugate can be detected in the urine (Miriam *et al.*, 1927).

## HIGHER HOMOLOGUES OF PHENYLACETIC ACID

The metabolic fate of a number of the higher homologues of phenylacetic acid has been studied, particularly in connection with the theory of the β-oxidation of fatty acids. The results of these studies are described in most text-books of biochemistry. The work of Knoop and of Dakin on these compounds is considered to be amongst the classics of biochemistry (cf. Dakin, *Oxidations and Reductions in the Animal Body*, London, 1922). It is therefore proposed not to deal with the fate of the phenyl-substituted aliphatic acids in great detail, but to cover the main points briefly for the sake of completeness.

The nature of the end-products of the metabolism of the phenyl-substituted fatty acids is dependent upon the number of carbon atoms in the aliphatic chain. Those containing an even number of carbon atoms are degraded through ketonic and unsaturated acids to phenylacetic acid, which appears in the urine as phenaceturic and phenylacetylglucuronic acids, whereas those containing an odd number of carbon atoms form benzoic acid which appears as hippuric and benzoylglucuronic acids.

$$Ph.(CH_2)_nCOOH \rightarrow Ph.CH_2COOH$$
$$Ph.(CH_2)_{n+1}COOH \rightarrow Ph.COOH$$

where $n$ = an odd number.

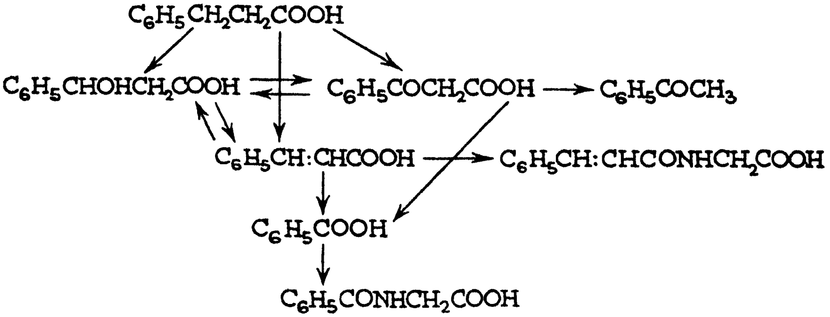
In the earlier work of Knoop and of Dakin, glucuronic acid conjugates were not taken into account, and in most cases only the glycine conjugates were considered. This led Leathes and Raper to question whether the fate of the phenylated acids could be entirely accounted for by the theory of $\beta$-oxidation. Quick (1928*a*) was therefore led to reinvestigate the fate of a number of these acids, and he was able to show that in dogs the major proportion of the phenyl-substituted fatty acids could be accounted for by the glycine and glucuronic acid conjugates eliminated. When benzoic acid is fed to dogs the ratio of benzoic acid excreted as benzoylglucuronide to that excreted as hippuric acid is 3 to 1, whereas in the case of phenylacetic acid the ratio, phenacetylglucuronide to phenaceturic acid, is 1 : 2. Quick showed that with phenyl-substituted acids containing an odd number of carbon atoms, the ratio of acid excreted conjugated with glucuronic acid to that eliminated as a glycine conjugate was the same as when benzoic acid was fed, i.e. 3 : 1. In the case of the acids with an even number of carbon atoms the ratio was 1 : 2 as for phenylacetic acid. This fact also applied to the unsaturated cinnamic and phenyl*iso*crotonic acids. Some of Quick's results are given in Table 11.

TABLE 11

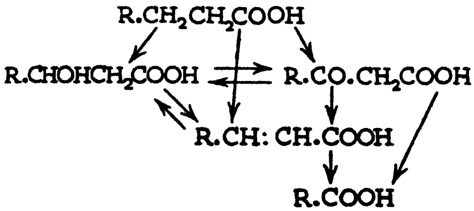
*Elimination of Phenyl-substituted Fatty Acids in the Dog (adapted from Quick,* 1928*a*)

| Acid | Side-chain | Benzoic or phenylacetic acid excreted combined with | |
|---|---|---|---|
| | | Glucuronic acid Percentage of total | Glycine Percentage of total |
| Benzoic | —$COOH$ | 74 | 26 |
| Phenylpropionic | —$CH_2CH_2COOH$ | 72 | 28 |
| Cinnamic | —$CH{:}CHCOOH$ | 70 | 29 |
| Phenylacetic | —$CH_2COOH$ | 30–35 | 65–70 |
| Phenylbutyric | —$CH_2CH_2CH_2COOH$ | 33 | 67 |
| Phenyl*iso*crotonic | —$CH{:}CHCH_2COOH$ | 31 | 69 |

**$\beta$-Phenylpropionic Acid.** That $\beta$-phenylpropionic acid is excreted as hippuric acid in dogs was first discovered by E. Salkowski and H. Salkowski (1882), but the investigation of its fate in detail was made a quarter of a century later by Dakin (1909*a*, cf. also Dakin 1908*a* and *b*). This acid is probably converted to hippuric acid according to the following scheme :—



Phenyl-β-hydroxypropionic acid, benzoylacetic acid (β-phenyl-β-keto-propionic acid) and acetophenone were all detected in the urine of dogs receiving phenylpropionic acid. Cinnamoylglycine (m.p. 192–193°) was also isolated and Dakin was not certain where this product fitted into the scheme for the metabolism of phenylpropionic acid since it was formed only in traces after the feeding of phenyl-β-hydroxypropionic acid and of cinnamic acid. Cinnamoylglycine itself is mainly excreted unchanged. However, later work suggests that unsaturated acids are formed in the animal body from saturated acids according to the following well-known scheme (see Dakin, 1922*a*) :—



## PHENYLATED ACIDS WITH UNSATURATED SIDE-CHAINS

Oxidation of phenylated acids with unsaturated side-chains occurs in the animal body with the production of benzoic or phenylacetic acid. They therefore resemble the corresponding saturated acids, and similarly whether benzoic or phenylacetic acid is produced depends on the number of carbon atoms in the side-chain. The degradation of the side-chain appears to be independent of the position of the double bond, for Dakin has shown that **phenyl-α:β-pentenic** and **phenyl-β:γ-pentenic** acids both give the same end-product as **δ-phenylvaleric acid.** A similar fate is undergone by **cinnamylidene acetic acid** which has two double bonds. Quick (1928*a*) has shown that **phenyl*iso*crotonic acid** which has a β:γ double bond undergoes oxidation in the α:β-position to give phenylacetic acid.

TABLE 12

| Acid | Side-chain | End-product |
|---|---|---|
| Cinnamic | $\mathrm{—CH{:}CHCOOH}$ | Benzoic acid |
| Phenyl*iso*crotonic | $\mathrm{—CH{:}CHCH_2COOH}$ | Phenylacetic acid |
| Phenyl-$\alpha{:}\beta$-pentenic | $\mathrm{—CH_2CH_2CH{:}CHCOOH}$ | Benzoic acid |
| Phenyl-$\beta{:}\gamma$-pentenic | $\mathrm{—CH_2CH{:}CHCH_2COOH}$ | ,, ,, |
| Cinnamylidene acetic | $\mathrm{—CH{:}CHCH{:}CHCOOH}$ | ,, ,, |

**Cinnamic Acid.** Mattschersky in 1863 observed that when cinnamic acid is fed to dogs there is an increase in the excretion of hippuric acid. Dakin found that the main product of cinnamic acid metabolism in the dog is hippuric acid. Small amounts of acetophenone, $\beta$-phenyl-$\beta$-hydroxypropionic acid and cinnamoylglycine are also excreted. Quick has also noted a large excretion of conjugated glucuronic acid and has assumed it to be benzoylglucuronide. Cinnamoylglycine itself is mainly excreted unchanged and does not give rise to hippuric acid. According to Dakin the mode of oxidation of cinnamic acid is as follows:—

$$\begin{tikzcd}
\mathrm{C_6H_5CH.CHCOOH} \arrow[rr, shift left] \arrow[dr] & & \mathrm{C_6H_5CHOH.CH_2COOH} \arrow[ll, shift left] \arrow[dl] \\
& \mathrm{C_6H_5COCH_2COOH} \arrow[d] & \\
& \mathrm{C_6H_5COCH_3} \arrow[d] & \\
& \mathrm{C_6H_5COOH} \arrow[d] & \\
& \mathrm{C_6H_5CONHCH_2COOH} &
\end{tikzcd}$$

**Cinnamylidene acetic acid** is of interest because it has two double bonds, and during its metabolism in the rabbit the occurrence of two consecutive $\beta$-oxidations can be demonstrated. According to Friedmann and Mai (1931) when this acid is injected subcutaneously into rabbits cinnamic acid, cinnamoylglycine, benzoic acid and hippuric acid are excreted, and therefore the degradation of the side-chain takes the following course:—

$$\mathrm{C_6H_5CH{:}CHCH{:}CHCOOH} \rightarrow \mathrm{C_6H_5CH{:}CHCOOH} \rightarrow \mathrm{C_6H_5COOH}$$

**Cinnamylidene malonic acid,** $\mathrm{C_6H_5CH{:}CHCH{:}C(COOH)_2}$, on the other hand, is not metabolised and is excreted unchanged (Dakin, 1909*b*).

## PHENYL HYDROXY ACIDS

Aromatic acids carrying a hydroxyl group in the side-chain are of interest since they are supposed to be formed during the metabolism of phenyl fatty acids, and in general, if the hydroxyl group is in the $\beta$-position of the side-chain, their metabolic fate follows the scheme due to

Dakin given on p. 112. Thus β-**phenyl**-β-**hydroxybutyric** and δ-**phenyl**-β-**hydroxyvaleric** acids are both oxidised to benzoic acid according to the β-oxidation theory (Dakin, 1909*c*).

When, however, the hydroxyl group is in the γ-position as in δ-**phenyl**-γ-**hydroxyvaleric acid** ($C_6H_5CH_2CHOHCH_2CH_2COOH$), the acid is scarcely attacked. The body finds great difficulty in oxidising this type of acid, and for the most part they are converted into lactones and excreted as such (Dakin).

Thus we see that when the hydroxyl group is in the β-position, the side-chain is oxidised *in vivo* to benzoic or phenylacetic acid, depending on the number of carbon atoms in the side-chain, whereas the aromatic nucleus remains unattacked. When the OH is in the γ-position both the side-chain and the aromatic nucleus are unattacked. When, however, the OH is in the α-position, it appears that in some cases both the ring and the side-chain are completely oxidised in the body, e.g. β-**phenyl**-α-**hydroxypropionic acid** ($C_6H_5CH_2CHOHCOOH$) (Knoop, 1905) and β-*p*-**chlorophenyl**-α-**hydroxypropionic acid** (Friedmann and Masse, 1910), whereas in others, e.g. mandelic acid ($C_6H_5CHOHCOOH$), the whole molecule is mainly excreted unchanged.

**Mandelic Acid.** Mandelic acid is of special interest since it was found as a metabolite of phenylglycine by Otto Neubauer during his well-known investigations on the biochemical oxidation of amino acids. Furthermore, it has recently found use as a bactericidal agent for the control of infection of the urinary tract and is a substitute for the old treatment of these infections by ketosis (Rosenheim, 1935; Helmholtz and Osterberg, 1936).

It had been known for some time that the urines of ketosis were bactericidal. The effective bactericidal factor in the urine of patients on a ketogenic diet is β-hydroxybutyric acid, which is bactericidal below *p*H 5·5. This acid, however, when given orally, is completely metabolised and thus does not appear in the urine in sufficient concentration to be bactericidal. Attempts to discover a therapeutic agent that might replace the ketogenic diet led to the clinical trial of mandelic acid, for this hydroxy acid is largely excreted unchanged and is at the same time bactericidal at *p*H 5–5·7 in concentrations of 0·25 to 1%. Mandelic acid has been successfully used, with ammonium chloride to produce the acid *p*H, in the treatment of urinary infections unassociated with urinary obstruction. Several other acids related to mandelic acid were also tested by Rosenheim, and it is of interest to note that benzoylacetic acid (see p. 116) is highly bactericidal *in vitro* but is ineffective *in vivo* because it is metabolised to other substances.

The main bulk of ingested mandelic acid is supposed to be excreted unchanged from the animal body. In 1867 Schultzen and Graebe stated that mandelic acid was readily converted into hippuric acid, but subsequent work showed this conclusion to be erroneous. A number of workers have expressed the opinion that when mandelic acid is formed in the body as the result of metabolic processes, it is excreted unchanged (cf. Knoop, 1905; Dakin, 1922). Schotten (1883–4), Knoop (1905) and Quick (1932) all showed that mandelic acid, when fed to dogs, is excreted unchanged. Nevertheless, more recent work (Wrede, 1938; Scholtz, 1938; Montenbruck, 1938) has shown that mandelic acid does undergo appreciable change both in man and in dogs. Some of the ingested acid is converted into benzoylformic acid (phenylglyoxylic acid), and when ammonium *r*-mandelate is fed (—)mandelic acid and benzoylformic acid can be recovered from the urine. When benzoylformic acid ($NH_4$ salt) is fed, (—)mandelic acid can be recovered, and it appears that an equilibrium may be set up between these substances:—

$$C_6H_5CHOHCOOH \leftrightarrows C_6H_5COCOOH$$

On feeding the separate optical isomerides results have been obtained which indicate that the *dextro* isomeride is more easily metabolised than the *laevo*, since when (—)mandelic acid is fed to man, 73% can be recovered as such and 7% as benzoylformic acid, whilst with (+)mandelic acid, 65% is recovered unchanged and 14% as benzoylformic acid. Neither acid was used in an optically pure state, so that the quantitative values obtained are open to question. The excretion of the optical isomerides of mandelic acid was further studied by Garry and Smith (1940), who injected *r*-mandelic acid intravenously into decerebrate cats and collected the urine fractionally. They found that the earlier samples of urine contained a marked excess of the *laevo* form, and in subsequent samples the preponderance of the *laevo* form became progressively less. Eventually the urine extracts became optically inactive and ultimately dextrorotatory, showing the excretion of a preponderance of the *dextro* acid. In long experiments the amounts of each form excreted nearly balanced, and some 80–90% of the injected acid was recovered. No trace of benzoylformic acid was detected, and when the pure *dextro* or *laevo* acid was injected both were recovered without alteration in stereochemical configuration. Garry and Smith have postulated that in the body there are probably optically active substances capable of forming compounds with mandelic acid without destruction of the stereochemical identity of the acid. The formation and destruction of these compounds may have different rates, depending on the nature of the mandelic acid, and consequently there may result a preferential excretion of (—)mandelic acid.

The question of the stereochemical relationships of mandelic acid has an important bearing on the well-known results of Neubauer and his co-workers on the fate of phenylglycine in the animal body. Neubauer showed that when *dl*-phenylglycine is fed to dogs (or perfused through dog livers) there can be recovered from the urine (—)phenylglycine, (—)mandelic acid, benzoylformic acid and hippuric acid. (+)Phenylglycine is metabolised with ease and benzoylformic acid and (—)mandelic acid are formed, whereas (—)phenylglycine is largely excreted unchanged. Neubauer interpreted these results as indicating that the primary step in the metabolism of phenylglycine was α-oxidative deamination with the production of benzoylformic acid, and since, as he supposed, (—)mandelic acid and (+)phenylglycine are stereochemically dissimilar, (—) mandelic acid must be formed in a secondary reaction, i.e. by the asymmetric reduction of benzoylformic acid, and not by direct oxidation of (+)phenylglycine :—

$$(+)C_6H_5CH(NH_2)COOH \longrightarrow C_6H_5COCOOH \longrightarrow (-)C_6H_5CHOHCOOH$$

Although Neubauer's conclusions have been questioned by Garry and Smith (1940), they are, nevertheless, correct, for (+)phenylglycine has an *l*-configuration and (—)mandelic acid a *d*-configuration (Kuna, Ovakimian and Levene, 1941 ; Freudenberg, 1933). Furthermore, Montenbruck (1940) has recently shown that administered (+)mandelic acid gives rise to some (—)phenylglycine *in vivo.*

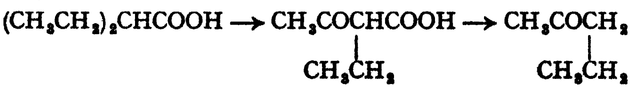
## PHENYL KETONIC ACIDS

It is a well-known fact that under certain circumstances the β-ketonic acid, acetoacetic acid may produce acetone *in vivo. Phenyl-β-ketonic acids* may also produce ketones in small amounts. The main product of their metabolism is either, benzoic or phenylacetic acid (depending on the number of carbon atoms in the side-chain according to the scheme given on p. 112). They may give rise to unsaturated acids and β-hydroxy acids as obligatory or incidental products in the degradation of the side-chain to —COOH or —$CH_2COOH$. The fate of **benzoylacetic acid** in the animal body has been studied (Dakin, 1911) and the main product of its metabolism is (—)phenyl-β-hydroxypropionic acid. Some hippuric acid is formed together with small amounts of acetophenone and cinnamoylglycine. Benzoylacetic acid obviously undergoes decarboxylation *in vivo* :—

$$C_6H_5COCH_2COOH \longrightarrow C_6H_5COCH_3 + CO_2$$

When benzoylacetic acid is formed during the metabolism of such acids as phenylpropionic or phenyl-β-hydroxypropionic, acetophenone always accompanies it in the urine. The formation of ketones, however, is not the main path of the catabolism of the phenyl-β-ketonic acids.

It may be of interest at this point to mention the case of **diethylacetic acid** ; when this acid is fed to an animal, it leads to the excretion of methylpropylketone (Blum and Koppel, 1911). The intermediate product is presumably the β-ketonic acid, α-ethylacetoacetic acid, which is decarboxylated to the ketone.



$$(CH_3CH_2)_2CHCOOH \longrightarrow CH_3CO\underset{\displaystyle CH_3CH_2}{\underset{|}{C}}HCOOH \longrightarrow CH_3CO\underset{\displaystyle CH_3CH_2}{\underset{|}{C}}H_2$$

*The α-ketonic acids* are important intermediates in the catabolism of the α-amino acids (Neubauer). In general, the phenyl-α-keto acids are oxidised *in vivo* to an acid with one less carbon atom, thus losing $CO_2$ in the process. The chain then presumably will follow the ordinary rules of β-oxidation, producing either benzoic or phenylacetic acid. In this case, however, a phenyl α-keto acid with an even number of carbon atoms in the side-chain will form benzoic acid, whilst one with an odd number will form phenylacetic acid, which is the opposite result to that found with the phenyl fatty acids. Thus benzoylformic acid is oxidised to benzoic acid and excreted as hippuric acid:—

$$C_6H_5COCOOH \rightarrow C_6H_5COOH$$

Some 36·7% of **p-chlorophenylpyruvic acid** is excreted as *p*-chlorophenaceturic acid (Friedmann and Maase, 1910).

$$p\text{-}ClC_6H_4CH_2COCOOH \rightarrow p\text{-}ClC_6H_4CH_2COOH \rightarrow p\text{-}ClC_6H_4CH_2CONHCH_2COOH$$

whereas **o-hydroxyphenylpyruvic acid** is mainly converted to *o*-hydroxyphenylacetic acid (Flatow, 1910).

The α-keto acids are also partially reduced to α-hydroxy acids; the formation of (—)mandelic acid from benzoylformic acid has already been mentioned.

*Phenyl γ-ketonic acids* probably give the same end-products as the corresponding unsubstituted phenyl fatty acids, for Knoop (1905) has shown that **γ-phenyl-γ-ketobutyric acid** (benzoylpropionic acid) is excreted as phenaceturic acid, and here both reduction of the keto group and a β-oxidation probably take place.

$$C_6H_5COCH_2CH_2COOH \xrightarrow{\text{reduction}} C_6H_5CH_2CH_2CH_2COOH \xrightarrow{\beta\text{-oxidation}} C_6H_5CH_2COOH$$

If the keto group were not reduced, then benzoic and hippuric acids would be expected, thus:—

$$C_6H_5COCH_2CH_2COOH \xrightarrow{\beta\text{-oxidation}} C_6H_5COCOOH \rightarrow C_6H_5COOH$$

Another γ-acid was studied by Knoop and Oeser, namely, **δ-benzyllevulinic acid** (ε-phenyl-γ-ketocaproic acid). The main product of the catabolism of this acid is phenaceturic acid:—

$$C_6H_5CH_2CH_2COCH_2CH_2COOH \rightarrow C_6H_5CH_2COOH$$

Small amounts of γ-phenyl-α-hydroxybutyric acid were also isolated, indicating probably the reduction of the keto group.

The nature of the end-products for the phenyl acids are summarised in Table 13.

## BRANCHED CHAIN PHENYL SUBSTITUTED ACIDS

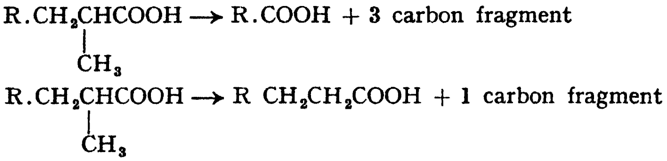
Studies on the metabolism of phenyl substituted acids containing branched chains have provided information which has an important bearing upon the theory of β-oxidation of the fatty acids.

α-Methyl substituted acids are of interest because they contain two β-carbon atoms and therefore two totally different pathways of oxidation are possible. They yield, however, the same end-products as the corresponding unsubstituted acids (see Kay and Raper, 1924; Kuhn and Livada, 1933). During the first stage in their oxidation either the α-methyl group may be oxidatively removed or β-oxidation of the chain may occur involving the removal of a three-carbon fragment:—

TABLE 13

*End-products of the Metabolism of Phenyl Substituted Acids*

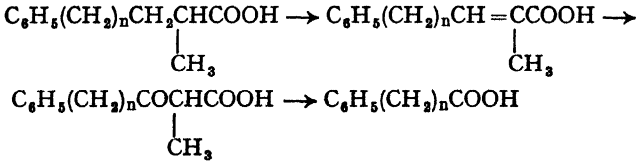
| Type of side-chain | End-product of catabolism | |
|---|---|---|
| | Number of carbon atoms in side-chain | |
| | Odd | Even |
| Saturated fatty acid | Benzoic acid | Phenylacetic acid |
| Unsaturated fatty acid | " " | " " |
| $\beta$-hydroxy acid | " " | " " |
| $\beta$-keto acid | " " | " " |
| $\gamma$-keto acid | " " | " " |
| $\alpha$-keto acid | Phenylacetic acid | Benzoic acid |
| $\alpha$-hydroxy acid | Some completely oxidised, some like $\alpha$-keto acids, some excreted unchanged. | |
| $\gamma$-hydroxy acid | Excreted unchanged. | |



$$\mathrm{R.CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCOOH \longrightarrow R.COOH + 3\ carbon\ fragment}$$

$$\mathrm{R.CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCOOH \longrightarrow R\ CH_2CH_2COOH + 1\ carbon\ fragment}$$

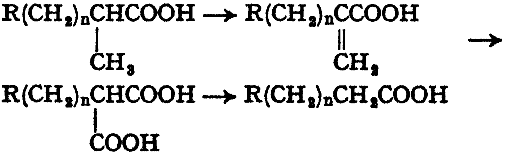
The recent work of Carter (1941) shows that the former scheme, i.e. removal of a 3C moiety, is probably correct, and an $\omega$-phenyl-$\alpha$-methyl acid is oxidised as follows:—



$$\mathrm{C_6H_5(CH_2)_nCH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HCOOH \longrightarrow C_6H_5(CH_2)_nCH{=}\underset{\displaystyle CH_3}{\underset{|}{C}}COOH \longrightarrow}$$

$$\mathrm{C_6H_5(CH_2)_nCO\underset{\displaystyle CH_3}{\underset{|}{C}}HCOOH \longrightarrow C_6H_5(CH_2)_nCOOH}$$

The earlier work of Kay and Raper (1922, 1924) on **hydratropic acid**[1] ($\alpha$-phenylpropionic acid), **$\alpha$-methyl-$\beta$-phenylpropionic acid** and **$\alpha$-methyl-$\gamma$-phenylbutyric acid** seemed to favour the second of these alternatives but repetition of this work by Carter showed that their conclusions were untenable. The mechanism suggested by Kay and Raper involves the formation of substituted malonic acids and $\alpha$-methylene acids as shown in the following scheme:—



$$\mathrm{R(CH_2)_n\underset{\displaystyle CH_3}{\underset{|}{C}}HCOOH \longrightarrow R(CH_2)_n\underset{\displaystyle CH_2}{\underset{\|}{C}}COOH \longrightarrow}$$

$$\mathrm{R(CH_2)_n\underset{\displaystyle COOH}{\underset{|}{C}}HCOOH \longrightarrow R(CH_2)_nCH_2COOH}$$

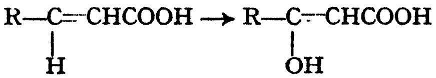
---

[1] Hydratropic acid, $C_6H_5CH(CH_3)COOH$, actually does not possess two $\beta$-carbon atoms.

However, Carter (1941) has shown that substituted malonic acids and α-methylene acids are largely recovered unchanged when fed (cf. cinnamylidene malonic acid, p. 113; Dakin, 1909). The body apparently has little ability to cleave $CO_2$ from the former acids.

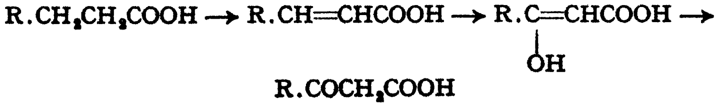
If the α-carbon atom of the acid carries a larger group than methyl, then the acid is not attacked *in vivo*. Thus **α-ethyl-** and **α-propyl-β-phenylpropionic** acids (Carter, 1941), **α-ethylcinnamic** (Fischer and Bielig, 1940, see p. 82) and **α-benzylcrotonic** acids (Kuhn and Livada, 1933) are largely excreted unchanged. In the substituted malonic acids, the α-substituent is COOH, which is larger than $CH_3$ and hence these acids are not metabolised.

The fate of the β-methyl-ω-phenyl acids is of interest since it throws new light on the mechanism of β-oxidation. Doubt has always existed regarding the position of the β-hydroxy and unsaturated acids in the well-known scheme of Dakin (see p. 112), and these products have often been regarded as side-products arising from the reduction of the β-keto acids. In the case of the β-hydroxy acid there is much evidence to show that this is the case (cf. Quick, 1928; Jowett and Quastel, 1935); it is very improbable that the β-hydroxy acid is an intermediate in the oxidation of saturated acids to β-keto acids. However, Carter (1941), basing his argument on the principle of vinylogy enunciated by Fuson (1935), has shown that the unsaturated acid plays a fundamental rôle in β-oxidation. The above principle states that if a vinyl group is interposed between the two groups, X and Y, the effect of Y on X transmitted through the —CH=CH— group is qualitatively unchanged and X behaves to some degree as if it were directly attached to Y. The compound X—CH=CH—Y is called a vinylogue of X—Y. The simple unsaturated acid is therefore a vinylogue of formic acid, thus:—



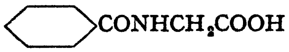
$$\mathrm{R{-}\underset{\overset{|}{H}}{C}{=}CHCOOH \longrightarrow R{-}\underset{\overset{|}{OH}}{C}{=}CHCOOH}$$

Since formic acid is readily oxidised, the α-β-unsaturated acid is also readily oxidised to form the enol form of the β-keto acid. Carter states, "It seems logical to assume, on this basis, that the unsaturated acid is an obligatory intermediate in β-oxidation; that the fundamental factor responsible for β-oxidation is the shift of the active point of the molecule to the β-carbon as a result of an initial desaturation of the fatty acid." He therefore proposes the following scheme for the mechanism of β-oxidation.

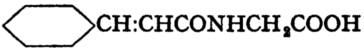


$$\mathrm{R.CH_2CH_2COOH \longrightarrow R.CH{=}CHCOOH \longrightarrow R.\underset{\overset{|}{OH}}{C}{=}CHCOOH \longrightarrow R.COCH_2COOH}$$

The principle of vinylogy also seems applicable in the synthesis of hippuric acid and cinnamoylglycine. Cinnamic acid is a vinylogue of benzoic acid and both these acids form glycine derivatives *in vivo* (see p. 113).



hippuric acid



cinnamoylglycine

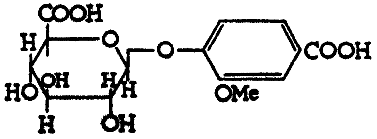
Another instance is to be found in the case of furoic acid and furfuracrylic acid, both of which form glycine derivatives *in vivo.*

## MISCELLANEOUS AROMATIC ACIDS

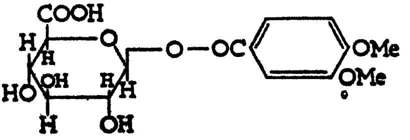
**Protocatechuic Acid** (3:4-dihydroxybenzoic acid). The urine of herbivorous animals is said to contain protocatechuic acid. According to Preusse (1878-9) the source of this acid is the plant material eaten. Protocatechuic acid may occur as such in the food or, more probably, it may be derived from the breakdown of tannin material and tannic acid present in the ingested plants. A rabbit kept on its normal vegetarian diet excretes material which gives the characteristic ferric chloride colour reaction for catechol derivatives, and both protocatechuic acid and catechol are detectable, but if the animal be kept on a diet of milk the amount of these compounds excreted gradually decreases to zero. Preusse kept a rabbit on a milk diet until the catechol reaction was negative and then fed it on the leaves of an ivy (*ampelopsis hederacea*), grass and hay. On this diet he was able to detect both protocatechuic acid and catechol in the urine.

Dog urine does not normally contain catechol derivatives (cf. Baumann, 1876). After feeding protocatechuic acid to dogs (Preusse, 1878–9) the urine was found to contain the unchanged acid, its ethereal sulphate and the ethereal sulphate of catechol. The occurrence of catechol in the urine indicates that protocatechuic acid undergoes decarboxylation *in vivo.* It is very probable that protocatechuic acid also forms a glucuronide.

**Vanillic Acid** (3-methoxy-4-hydroxybenzoic acid). About half (56%) the acid fed is excreted in the free state and about a quarter (27%) is conjugated. Of the conjugated acid eliminated about two-thirds (16% of the dose) appears as glucurovanillic acid (*o*-methoxy-*p*-carboxyphenylglucuronide) and just over a third (11% of the dose) forms an ethereal sulphate. A demethylation also takes place to a slight extent and a small amount of vanillic acid is excreted probably as protocatechuic acid. The acid does not form an ester glucuronide in the rabbit and no glycine conjugate can be found. Vanillic acid is therefore unlike most aromatic acids, in which the carboxyl group is involved in conjugation (Sammons and Williams, 1941).



glucurovanillic acid



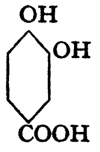
veratroylglucuronide

**Veratric Acid** (3:4-*dimethoxybenzoic acid*). The metabolisms of the monomethyl and the dimethyl ethers of protocatechuic acid in the rabbit are strikingly different, presumably because in vanillic acid there is a free hydroxyl group, whilst in veratric acid this hydroxyl is blocked by a methyl group. In vanillic acid conjugation is effected through the phenolic hydroxyl, whereas in veratric acid it is confined to the carboxyl group at the opposite end of the molecule. Sammons and Williams (1946) found about 41% of the acid fed to rabbits to be excreted in the free state and 28–29% combined, and some 2% undergoes double demethylation to a catechol derivative which is excreted as an ethereal sulphate. The whole of the combined veratric acid appears in the urine as veratroylglucuronide (m.p. 169–170°; $[\alpha]_D - 13{\cdot}1°$ in water), which is very similar to benzoylglucuronide in properties; it reduces the common sugar reagents and its rotation changes in the presence of alkali. No glycine conjugate is found.

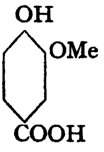
**Piperonylic acid** (3:4-methylenedioxybenzoic acid) when fed to rabbits gives a reducing urine, and it is probable that a glucuronide (piperonyloylglucuronide) is excreted. It also forms a glycine conjugate, piperonyloylglycine (3:4-methylenedioxyhippuric acid, m.p. 178°) which can be isolated from the urine (Williams, unpublished results).

Piperonylic acid is apparently produced and excreted as such during the metabolism of safrole[1] and *iso*safrole in the dog (see also piperonal, p. 88) (Heffter, 1895).

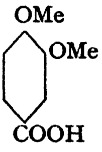
The formulae for the compounds mentioned above are collected below :—



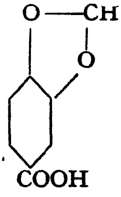
protocatechuic acid



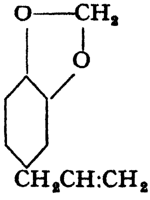
vanillic acid



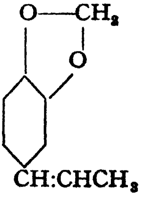
veratric acid



piperonylic acid



safrole



*iso*safrole

**The Naphthoic Acids.** The fate of both α- and **β-naphthoic acids** in the animal body has been investigated. According to Cohn (1894*a*) α-naphthoic acid when fed to rabbits is excreted unchanged and no conjugated form can be detected, but in the dog it forms a small amount of α-naphthuric acid (α-naphthoylglycine, m.p. 155°). Quick (1932) found that α-naphthoic acid forms an appreciable amount of ester glucuronide (α-naphthoylglucuronide, not isolated) in the dog, but only very small amounts of the glycine conjugate, and in this respect it behaves like an *ortho* substituted benzoic acid. β-Naphthoic acid, on the other hand, forms no conjugate in the dog, whereas in the rabbit it forms a small amount of *β*-naphthuric acid (m.p. 169–170°)

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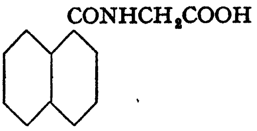
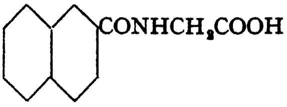
[1] Main component of sassafras oil.

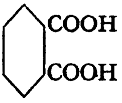


*α*-naphthuric acid

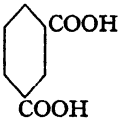


*β*-naphthuric acid

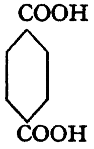
**Benzenedicarboxylic Acids.** The three benzene dicarboxylic acids, **phthalic,** *iso***phthalic** and **terephthalic** acids are largely excreted unchanged. Phthalic acid, when injected subcutaneously into dogs (Pohl, 1909; Pribram, 1904), can be recovered unchanged in the urine almost quantitatively. This is true also in man (Shemiakin and Schukina, 1944). Some 75% of both *iso*- and terephthalic acids can be recovered from the dog, but the rest may be oxidised completely (Porcher, 1908). These acids do not form glycine conjugates.



phthalic acid



*iso*phthalic acid



terephthalic acid

TABLE 14

*Biosynthetic Glucuronides of Aromatic Acids*

| Glucuronide | M.P. | Specific rotation $[\alpha]_D$ in water | References |
|---|---|---|---|
| Benzoyl | 184–185° | − 26·5° | Goebel (1938) |
| *p*-Dimethylaminobenzoyl | — | − 12° (dilute HCl) | Jaffe (1905) |
| 3:4-Dimethoxybenzoyl (Veratroyl) | 169–170° | − 13·1° | Sammons and Williams (1946) |
| *p*-Glucuronosidobenzoyl (*p*-hydroxybenzoic acid diglucuronide) | decomp. above 200° | − 15 to − 16° | Quick (1932*b*) |
| Diphenylacetyl | 180–185° (decomp.) | − 34·75° | Miriam, Wolf and Sherwin (1927*a*) |

TABLE 15

*Biosynthetic Substituted Hippuric Acids*

| Substituted hippuric acid | M.P. | Reference |
|---|---|---|
| No substituent | 187° (190°) | |
| *o*-methyl | 162·5° | Gleditsch and Moeller (1888) |
| *m*-methyl | 139° | ,, ,, ,, ,, |
| *p*-methyl | 161–162° | ,, ,, ,, ,, |
| (toluric acids) | | cf. Schultzen and Naunyn (1867) |
| *o*-fluoro | 121° | Coppola (1883) |
| *m*-fluoro | 152–153° | ,, ,, |
| *p*-fluoro | 161° | ,, ,, |
| *o*-chloro | 176° | Hildebrandt (1903) ; Novello *et al.* (1926) |
| *m*-chloro | 144° | ,, ,, ,, ,, ,, |
| *p*-chloro | 143° | ,, ,, ,, ,, ,, |
| *o*-bromo | 153° | ,, ,, ,, ,, ,, |
| *m*-bromo | 183° | ,, ,, ,, ,, ,, |
| *p*-bromo | 159–162° | ,, ,, ,, ,, ,, |
| *o*-iodo | 170° | Novello, Miriam and Sherwin (1926) |
| *m*-iodo | 167–169° | ,, ,, ,, ,, ,, |
| *p*-iodo | 188–190° | ,, ,, ,, ,, ,, |
| *o*-hydroxy (salicyluric acid) | 170° | Quick (1932*b*) |
| *m*-hydroxy | 182–183° | ,, ,, |
| *p*-hydroxy | 227° | ,, ,, |
| *m*-nitro | 165–167° | Cohn (1893) |
| *p*-nitro | 179° | Jaffe (1874) |
| *m*-amino | 194° | Salkowski (1882) |
| *m*-methoxy | 122° | Quick (1932*b*) |
| *p*-methoxy (anisuric acid) | 170° | ,, ,, |
| 3:4-methylenedioxy (piperonyloylglycine) | 178° | Heffter (1895) |
| α-naphthoylglycine (α-naphthuric acid) | 155° | Cohn (1894*a*) |
| β-naphthoylglycine (β-naphthuric acid) | 169–170° | Cohn (1894*a*) |

## TABLE 16

*Biosynthetic Glycine Derivatives of Phenylacetic and Cinnamic Acids*

| Compound | M.P. | Reference |
|---|---|---|
| Phenacetylglycine (phenaceturic acid) | 142–143° | Sherwin (1917) |
| *o*-Chlorophenaceturic acid | 134–135° | Cerecedo and Sherwin (1923–4) |
| *p*-Chlorophenaceturic acid | 165° | ,, ,, ,, ,, |
| *p*-Bromophenaceturic acid | 160–165° | ,, ,, ,, (1924–5) |
| *p*-Nitrophenaceturic acid | 172–173° | Sherwin and Helfand (1919) |
| *p*-Aminophenaceturic acid | sinters at 200° | Cerecedo and Sherwin (1923–4) |
| Cinnamoylglycine | 193° | Dakin (1909*a*) |
| β-Methylcinnamoylglycine | 145° | Kuhn and Livada (1933) |

## TABLE 17

*Biosynthetic Ornithuric Acids*[1] *from Hens*

| Compound | M.P. | Reference |
|---|---|---|
| α:δ-N:N′-dibenzoylornithine (ornithuric acid) | 182° (188–189°) | Jaffe (1877) |
| α:δ-N:N′-diphenacetylornithine | 139–139·5° | Totani (1910) |
| α:δ-N:N′-di-(*p*-nitrophenacetyl)-ornithine | 184–185° | Sherwin and Helfand (1919) |
| α:δ-N:N′-difuroylornithine | 186° | Jaffe and Levy (1888) |

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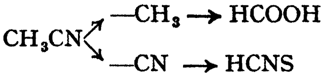
[1] These acids are optically active, but their rotations have only been accurately recorded for synthetic preparations.

# *Chapter Eight*

# The Metabolism of Organic Cyanides

## ALIPHATIC NITRILES

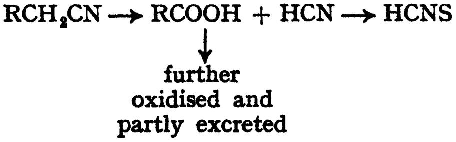
ALIPHATIC cyanides are metabolised in the animal body with the splitting off of HCN which leads to an increase in the excretion of thiocyanates. The CN group may also be hydrolysed to some extent to COOH, for according to Giacosa (1883), **acetonitrile** and **propionitrile** are converted in the dog to acetic and propionic acids respectively. This is not, however, borne out by the more careful work of Lang (1894), who showed definitely that acetonitrile was converted in the dog into thiocyanic and formic acids. Some 20% of the CN group of acetonitrile could be accounted for by the thiocyanate excreted in the urine, thus :—



$$CH_3CN \begin{array}{l} \nearrow -CH_3 \rightarrow HCOOH \\ \searrow -CN \rightarrow HCNS \end{array}$$

The mechanism of the conversion of the methyl radicle of acetonitrile to formic acid is not clear. The conversion of the CN group to CNS is discussed later. **Propio, butyro-** and **capronitriles** given in small doses also result in the excretion of thiocyanates, but the fate of the alkyl residue is uncertain. According to Lang (1894) these three compounds are highly toxic bodies, whereas acetonitrile is of relatively lower toxicity.[1] The high toxicity of propio-, butyro- and capronitriles may be due to their possessing a specific toxic action as well as a high rate of breakdown to hydrogen cyanide ; in support of this Lang points out that the ethyl, propyl and butyl groups are often more rapidly oxidised *in vivo* than are methyl groups (cf. the aliphatic alcohols, Chapter Two). It is probable that the toxicities of these nitriles are dependent on the rate at which HCN is split off, and in this way their toxic properties may be closely related to their metabolism.

The changes undergone by aliphatic nitriles in the animal body can probably be represented as follows :—



$$\begin{array}{c} RCH_2CN \rightarrow RCOOH + HCN \rightarrow HCNS \\ \downarrow \\ \text{further oxidised and partly excreted} \end{array}$$

---

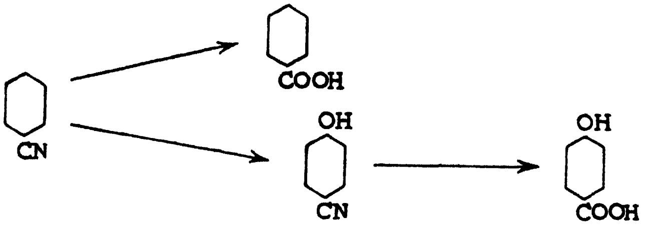
[1] *Toxicity for Rabbits* (Francis and Fortescue-Brickdale, 1908)

| | |
|---|---|
| Acetonitrile | 0·13 g./kg. |
| Propionitrile | 0·065 ,, |
| Butyronitrile | 0·010 ,, |
| *Iso*butyronitrile | 0·009 ,, |
| *Iso*valeronitrile | 0·045 ,, |

## AROMATIC NITRILES

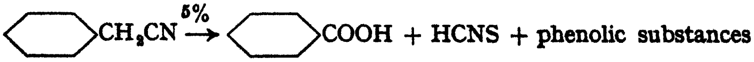
Adeline, Cerecedo and Sherwin (1926) are of the opinion that the toxicity of the aromatic cyanides is not related to the rate at which they split off HCN, but is a specific property of the cyanide molecule as a whole. Information regarding the metabolism of aromatic nitriles is very scanty, and except in one or two cases no clear picture can be drawn. The above workers obtained very variable results and only a small percentage of the dose could be accounted for by the metabolites excreted in the urine. In some cases there was a slight conversion of the CN group to COOH, whereas in others small increases in ethereal sulphate and thiocyanate output were observed.

**Benzonitrile.** In the dog Giacosa (1883) found that small amounts of benzoic acid were excreted after feeding benzonitrile. The benzene ring is also oxidised since an increase in organic sulphates was observed, and this was confirmed by Baumann (1883*a*). The latter worker actually isolated hydroxybenzoic acids from the urine of a dog which had received 25 g. of benzonitrile over several days ; 0·27 g. *p*-hydroxybenzoic acid and 0·114 g. salicylic acid were obtained. It seems probable that at least part of the benzonitrile molecule may undergo hydrolysis and oxidation according to the following scheme :—

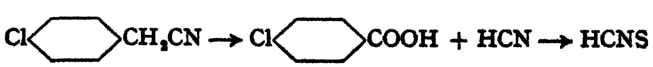


Whether oxidation of the ring to a hydroxybenzonitrile or hydrolysis of the cyanide group to carboxyl is the primary reaction has yet to be decided, but from the fact that benzoic acid does not give rise to hydroxybenzoic acid *in vivo*, it is plausible to suggest that the former is the primary change. Adeline *et al.* (1926) failed to detect metabolites of benzonitrile or of its **2:4-dichloro** derivative, but showed that the *p*-**chloro** derivative was in large part excreted unchanged in the faeces and sweat. The dog tolerated 2:4-dichlorobenzonitrile well.

**Benzylcyanide** gives rise to small amounts of benzoic acid and causes an increased output of ethereal sulphates and thiocyanate, although the amounts excreted do not account for more than 5% of the substance fed :—



*p*-**Chlorobenzylcyanide** undergoes oxidation in the dog with the production of *p*-chlorobenzoic acid and the cyanide group is partly excreted as thiocyanate.



From the urine of a dog which had received one gram of this cyanide Adeline *et al.* (1926) were able to isolate 0·8 g. of *p*-chlorobenzoic acid. If the CN group were hydrolysed, one would expect the excretion of *p*-chlorophenylacetic acid, so it appears that the CN group is split off as a whole, and this is followed by oxidation of the $CH_2$ group. Adeline *et al.* (1926) drew attention to the interesting observation that whereas *p*-chlorobenzoic acid in the dog gives rise to the excretion of considerable amounts of *p*-chlorohippuric acid, no glycine conjugate can be detected when *p*-chlorobenzonitrile is fed, although the major proportion of the nitrile must be metabolised to *p*-chlorobenzoic acid. ***p*-Nitrobenzylcyanide** is extremely insoluble and is consequently of low toxicity. In the dog it is mainly excreted unchanged in the faeces, although a small amount of *p*-nitrobenzoic acid appears in the urine. The fate of the absorbed part is thus similar to that of benzylcyanide. In the rabbit *o*-**nitrobenzylcyanide** gives rise to a marked increase in the excretion of ethereal sulphates, indicating oxidation to a phenol, whereas in the dog a small amount undergoes oxidation to *o*-nitrobenzoic acid

It appears probable from what has been said above that when the CN group is attached directly to the aromatic ring, it is at least partly hydrolysed *in vivo* to COOH and presumably, ammonia, whereas in those cases where the CN group is separated from the ring by a $CH_2$ group, it is split off as HCN, which is then detoxicated to thiocyanate. With aliphatic compounds the second type of change may take place, but conversion to a fatty acid and ammonia cannot be definitely ruled out (i.e. $RCH_2CN \rightarrow RCH_2COOH + NH_3$). The above can be summarised as follows: –

$$RCH_2CN \rightarrow RCOOH + HCN \rightarrow HCNS$$
$$ArCN \rightarrow ArCOOH + (NH_3\ ?)$$
$$ArCH_2CN \rightarrow ArCOOH + HCN \rightarrow HCNS$$

where R = Alkyl group
and Ar = Aromatic group

## The Enzymic Conversion of Cyanide to Thiocyanate

Thiocyanates are said to be normally excreted by the animal organism (Nichols, 1925) and human saliva contains on an average 0·01% of CNS′ (see Shohl, 1939, or Hawk and Bergeim, 1939), whereas in normal blood the amount is about 1·31 mg. KCNS per 100 c.c., the values for habitual smokers being well above this figure (Trasoff and Schneeberg, 1944). The formation of thiocyanic acid *in vivo* is an enzymic process involving the enzyme rhodanese (Lang, 1933). This enzyme occurs in nearly all animal tissues except blood and muscular tissue; in the dog it reaches its highest concentration in the suprarenal glands, the next richest source being the liver, which, in view of its size compared with the suprarenals, contains the major part of the body's rhodanese supply. The amount of the enzyme found in the livers of various species of animals is variable for, according to Lang (1933), dried liver powder from the frog contains more than fifty times as much of the enzyme per 100 g. as does dog liver, whereas the rabbit has twenty times, the cow ten times and the human being three times as much as the dog. The low concentration of rhodanese in dog liver compared with rabbit liver is reflected in the results of Mukerji and Smith (1943), who found injected cyanide to be converted into thiocyanate much less efficiently in the dog than in the rabbit. The latter animal converts intravenously or subcutaneously injected cyanide almost wholly into thiocyanate, which is completely excreted within 24–48 hours. Dog liver rhodanese is thermolabile, being inactive at 56° and has optimum activity at about body temperature (38°) and *p*H 8·3. The enzyme is specific for hydrogen cyanide which it converts very rapidly in the presence of sodium thiosulphate or colloidal sulphur into thiocyanic acid. Certain sulphur-containing organic compounds such as cystine, cysteine, glutathione and thioethanolamine, can also act as substrates for this enzyme, but the amount of thiocyanic acid formed in the presence of these is only 1/100 to 1/1000 of that formed with thiosulphate or colloidal sulphur. The enzyme in the presence of thiosulphate or colloidal sulphur does not form thiocyanate *in vitro* from organically bound CN groups, such as those found in acetonitrile, propionitrile, cyanacetic acid and cyanamide. This property indicates that the excretion of thiocyanates after the administration of organic nitriles, results not from a direct action on the nitrile itself but from HCN which has first been removed from the nitrile molecule by another mechanism. Thiocyanate is also formed from sodium sulphide as a source of sulphur, but only in the presence of oxygen. No thiocyanate is formed in the absence of oxygen and it appears that sulphide is not directly utilisable, but is first oxidised by oxygen to elementary sulphur, which then reacts with HCN in the presence of the enzyme.

According to Lang (1933) the reaction between HCN and $Na_2S_2O_3$ catalysed *in vitro* by rhodanese does not require oxygen and may be represented as follows :—

$$HCN + Na_2S_2O_3 = HCNS + Na_2SO_3$$

If this reaction took place in the body, the sulphite would be rapidly oxidised to sulphate. The compound or compounds which provide sulphur for the detoxication of cyanide *in vivo* are not known. Lang (1933) points out that thiosulphate has never been detected as a metabolic product in man, but the more recent work of Vassel, Partridge and Crossley (1944) has shown that dogs excrete thiosulphate as long as they are fed, but if food is withheld thiosulphate excretion ceases in both sexes. These workers used thoroughbred bull terriers and found a marked difference in the thiosulphate output of males and females. The bitches excreted 2–15 mg. thiosulphate-S in 24 hours, whereas the dogs excreted 50–125 mg.

In the treatment of cyanide poisoning it has been found that the intravenous injection of sodium nitrite followed by sodium thiosulphate is more effective than either substance alone. Both substances are effective against cyanide, but when used together there is not only a summation but a definite potentiation of action (Chen, Rose and Clowes, 1944). Apparently sodium nitrite first reacts with haemoglobin to form methaemoglobin, which then combines with HCN to form non-toxic cyanmethaemoglobin (Chen *et al.*, 1944). It will be recalled that the blood and musculature do not contain rhodanese, and thiosulphate is likely to have no action on HCN in the bloodstream. When the HCN reaches other tissues it is converted to non-toxic thiocyanate by thiosulphate in the presence of rhodanese. It is also possible that cyanmethaemoglobin formed in the blood sets free HCN in other tissues, which is then detoxicated by thiosulphate catalysed by rhodanese. In fact, Etteldorg and Morrison (1940) have shown that cyanmethaemoglobin on injection into dogs is converted to functional haemoglobin at the rate of 1·02–1·76 vols. % per hour, methaemoglobin probably being formed as an intermediate.

The conversion of hydrogen cyanide to thiocyanic acid is a true detoxication, for in the process there is a 200-fold reduction in toxicity. The occurrence of a highly specific enzyme in tissues and of thiocyanate in the excretions indicates the possibility that hydrogen cyanide is formed during normal metabolism. It is doubtful, however, whether the mechanism can deal with quantities of hydrogen cyanide very much greater than those formed during normal intermediary metabolism. The conversion of hydrogen cyanide to thiocyanic acid can be regarded as a true detoxication mechanism in the limited sense that it deals with the quantities of toxic body formed during normal metabolism or eaten in a normal diet (cf. herbivores) and not with abnormal quantities introduced into the organism artificially.

## *Chapter Nine*

# The Metabolism of the Aromatic Nitro, Amino and Azo Compounds

THE aromatic nitro, amino and azo compounds form a most important group of nitrogenous substances of practical use to man. In this group we find a large variety of therapeutic agents and a wide range of dyes, whereas the majority of explosives are nitro compounds. As these compounds are manufactured on a very large scale, their industrial production often entails health hazards for those persons employed in their manufacture. A knowledge of the fate of these compounds in the animal body is therefore of the greatest importance for the understanding of the mechanism whereby these compounds produce their toxic effects on man.

### AROMATIC NITRO COMPOUNDS

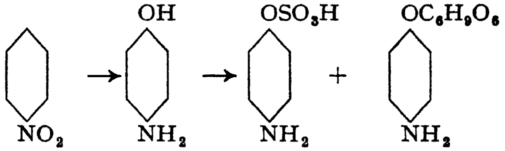
Although our knowledge of the fate of aromatic nitro compounds is not very extensive, even less is known about that of aliphatic nitro compounds. Before dealing with the aromatic compounds it may be useful for the purposes of comparison to discuss the meagre information available concerning the aliphatic nitro group. It appears probable that the aliphatic nitro group is set free as nitrite, for Scott (1942) has shown that **nitroethane** on intravenous injection into rats is partly converted to acetaldehyde and nitrite. The conversion of the nitro group to nitrite is equivalent to a reduction, $—NO_2 \rightarrow —ONO$. The nitrite formed is rapidly converted to nitrate, for in rabbits receiving nitroethane in air by means of a tracheal cannula, the blood $NO_2'$ is always low owing to the rapid conversion to $NO_3'$; the latter is only slowly excreted (Scott, 1943). There is no record that the aliphatic nitro group may be reduced *in vivo* to a hydroxylamine, —NHOH, or to an amine, $—NH_2$. **Hydroxylamine** itself is said to produce a trace of nitrite in the urine of animals (Cuisa and Luzzatto, 1908), a fact which indicates that hydroxylamine is oxidised *in vivo.*

The aromatic nitro group is reduced in the animal body to the corresponding amino group. Chemically the reduction of the $—NO_2$ group takes place in three stages, involving the intermediate formation of the nitroso and hydroxylamino groups.

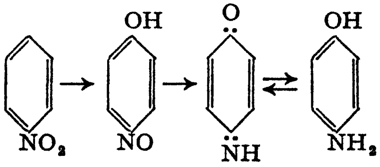
$$—NO_2 \rightarrow —NO \rightarrow —NHOH \rightarrow —NH_2$$

It is probable that the biological reduction also takes the same course, since the hydroxylamino, but not the nitroso, stage has been observed. It could be expected that if the nitroso compound were formed, such products as azoxy and azo derivatives would also be formed; there is, however, no experimental proof of the biological formation of such compounds. It is also probable that the hydroxylamine may rearrange to an aminophenol so that the formation of an amino compound may result either by direct reduction of the hydroxylamino group or by rearrangement of the hydroxylamine; probably, both reactions take place.

**Nitrobenzene.** The fate of nitrobenzene in the animal body has been the subject of numerous investigations, but opinions are divided as to the sequence of changes involved in its metabolism. The products found in the urine after its administration are small amounts of unchanged nitrobenzene and large amounts of *p*-aminophenol and its compounds with glucuronic and sulphuric acids (Meyer, 1905).

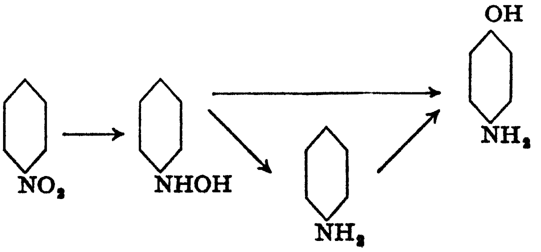


The mechanism whereby nitrobenzene is converted into *p*-aminophenol is still a matter of controversy. In order to explain the toxic action of nitrobenzene on blood and the formation of methaemoglobin, Heubner (1913, 1914) suggested the following mechanism:—



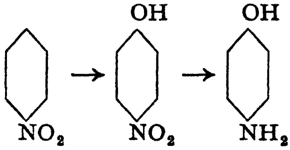
*p*-Nitrosophenol, quinoneimine and *p*-aminophenol are all powerful methaemoglobin-formers (Heubner and Meier, 1923).

Meyer suggested that since *β*-phenylhydroxylamine is converted to *p*-aminophenol *in vivo*, it also may be an intermediate. In this reaction the hydroxylamine probably rearranges



to *p*-aminophenol, although there is an alternative route whereby the hydroxylamine could be reduced to aniline and then oxidised to *p*-aminophenol. In plant tissues it is known that the hydroxylamine can form aniline, for Neuberg and Welde (1914) have shown that fermenting yeast can reduce nitrobenzene, nitrosobenzene and phenylhydroxylamine to aniline. It is also known that *p*-aminophenol is a product of aniline metabolism, but aniline has not been detected as a metabolite of nitrobenzene in the animal. The position of *β*-phenylhydroxylamine is more certain since Channon, Mills and Williams (1944) have actually isolated 4-hydroxylamino-2:6-dinitrotoluene from the urine of rabbits receiving 2:4:6-trinitrotoluene orally.

Another scheme has been suggested by the work of Meyer on *p*-nitrophenol. This compound is converted into *p*-aminophenol in the rabbit, and it could therefore be an intermediate in the conversion of nitrobenzene to *p*-aminophenol (cf. Heubner's scheme above) :—



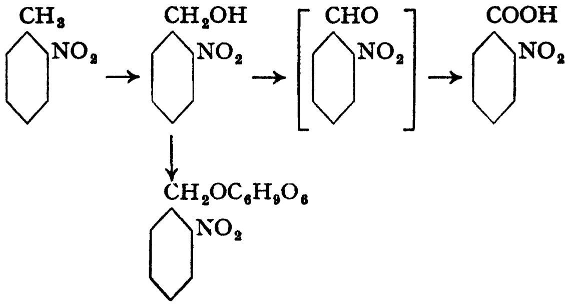
The formation of nitrosobenzene as the precursor of phenylhydroxylamine whilst being probable has not as yet received experimental confirmation. If it were formed, the formation of azoxybenzene and azobenzene would not be unexpected ; these compounds, however, have not, as yet, been detected.

Further work is necessary, particularly on the quantitative side, before the mechanism of the conversion of nitrobenzene to *p*-aminophenol can be understood fully.

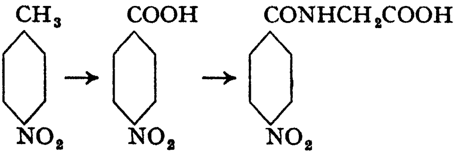
**Dinitrobenzene.** Little is known regarding the fate of the polynitrobenzenes. The three dinitrobenzenes are probably partly reduced *in vivo*, for Lipschitz and Osterroth (1924) have shown that all three are reduced in frog muscle pulp, the *ortho* compound being readily reduced, the *meta* and *para* with difficulty. *m*-**Dinitrobenzene** is of great practical importance in the dye and explosives industry and consequently it has received more attention than its isomerides. Little definite information is available regarding its metabolism in the animal. According to White, Hay and Orsman (1902) and Koelsch (1918) it is excreted partly unchanged and partly as changed products which may include *m*-nitroaniline, since Lipschitz obtained evidence that, *in vitro*, blood reduces it to *m*-nitrophenylhydroxylamine.

## THE NITROTOLUENES

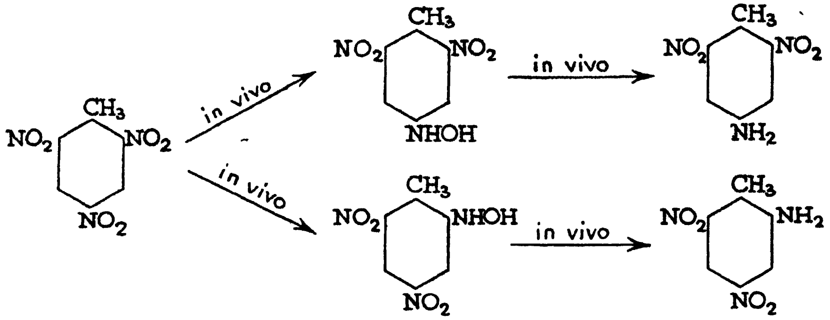
The fate of the nitrotoluenes in the animal body was studied at an early date by Jaffe (1874 ; 1878–9). *o*-**Nitrotoluene** is converted in dogs to *o*-nitrobenzyl alcohol and *o*-nitrobenzoic acid. The former compound is excreted to the extent of 25% as *o*-nitrobenzylglucuronide which can be isolated as a complex with urea. The *o*-nitrobenzoic acid excreted corresponds to 10% of the dose fed. The production of these two substances represents two stages in the oxidation of the methyl group of *o*-nitrotoluene ; a third stage which was not detected is probably that of *o*-nitrobenzaldehyde. Jaffe records no changes in the nitro group, although it is probable that it is reduced to some extent. As far as Jaffe's results go, the metabolism of *o*-nitrotoluene can be represented as follows:—



A glucuronide apparently is not formed in the case of ***p*-nitrotoluene,** which in dogs is mainly converted into *p*-nitrobenzoic acid and excreted as *p*-nitrohippuric acid. Although Jaffe does not record the finding of any reduction products it seems likely, in view of the fact that both *p*-nitrobenzoic acid and *p*-nitrobenzaldehyde are converted to *p*-aminobenzoic acid, that *p*-nitrotoluene is also excreted as *p*-aminobenzoic acid.

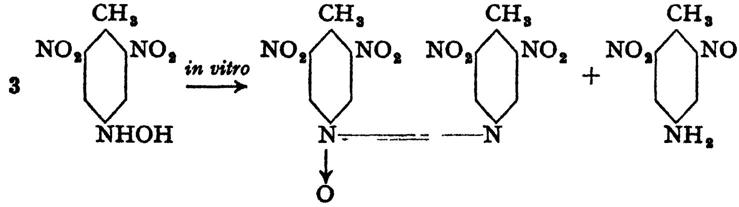


**2:4:6-Trinitrotoluene** (α-**T.N.T.**). By far the most important of the nitrotoluenes is the high explosive T.N.T. The fact that workers engaged in its manufacture often show toxic symptoms and occasionally die stimulated much investigation of its fate in the animal body. The earlier work (cf. Moore, 1917; Dale, 1921; Voegtlin, Hooper and Johnson, 1920) on the metabolism of T.N.T. yielded little definite information, except to show that T.N.T. was reduced to a dinitroaminotoluene of undefined structure and that tetranitroazoxytoluene could be isolated from the urine of T.N.T. workers and experimental animals receiving T.N.T. orally or by injection. The more recent work of Channon, Mills and Williams (1944) has now given a clearer picture of the metabolism of T.N.T. in experimental animals. They found that urine from T.N.T.-fed rabbits contained a number of metabolites and they were able to identify and isolate three of them. These were 2:6-dinitro-4-hydroxylaminotoluene, 2:6-dinitro-4-aminotoluene and 2:4-dinitro-6-aminotoluene. These results show that at least two of the three nitro groups of T.N.T. are changed in the rabbit and that a hydroxylamine is formed as an intermediate stage in the reduction of the $NO_2$ group to $NH_2$. The 4-amino compound was obtained in greater yield than the 6-amino, indicating that the *para*-nitro group is more easily reduced than the two *ortho* groups. The reduction of T.N.T. to dinitroaminotoluenes can therefore be represented as follows:—

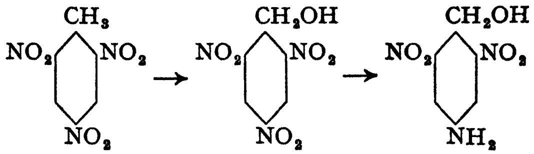


24–30% of the T.N.T. administered appears as compounds containing a diazotisable amino group, but these are not necessarily made up entirely of dinitroaminotoluenes since metabolites which have undergone both oxidation and reduction have to be taken into account. The above scheme omits any reference to an intermediate nitroso compound; whilst dinitronitrosotoluene is a probable metabolite, no evidence has been obtained that it is formed. Products of the possible rearrangement of the hydroxylamino compound such as dinitroaminocresols were not detected, and the formation of the amino compound is probably the result of the direct reduction of the hydroxylamino group.

A number of investigators have claimed that 2:2′:6:6′-tetranitro-4:4′-azoxytoluene is a metabolite of T.N.T., and it has been isolated in crystalline form from the T.N.T. urine of humans and experimental animals. Its formation from dinitrohydroxylaminotoluene is easily envisaged and *in vitro* this conversion is exceedingly easy to carry out. Channon *et al.* (1944) have shown that it is not present in freshly voided urine and it is in fact an artefact produced from the hydroxylamino compound during isolation procedures.

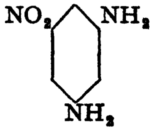


T.N.T. also causes an increased output of glucuronic acid, and, in rabbits, Channon *et al.* (1944) have shown that nearly one-half of the T.N.T. administered can be accounted for as a conjugated glucuronide. The nature of the glucuronide or glucuronides is uncertain, but the evidence points to the existence of trinitrobenzyl or dinitroaminobenzyl glucuronide, or both, in T.N.T. urine from rabbits. The postulate that 2:4:6-trinitrobenzyl alcohol is a metabolite of T.N.T. is strengthened first of all by the analogous case of *o*-nitrotoluene which was shown by Jaffe (1878–9) to be converted to *o*-nitrobenzyl alcohol in the dog, and secondly by the fact that of a large number of nitro compounds tested only trinitrobenzyl alcohol will cause the excretion of the red pigment which is found in the T.N.T. urine of rats and rabbits (Channon *et al.*, 1944). This **trinitrobenzyl alcohol** when fed to rabbits is reduced to a diazotisable substance which may be a dinitroaminobenzyl alcohol. These facts, therefore, point to the following possible oxidation mechanism during the metabolism of T.N.T.



Both dinitrohydroxylaminotoluene and trinitrobenzyl alcohol are tissud poisons and form methaemoglobin *in vitro*, the hydroxylamino compoune being particularly powerful in this respect. The formation of these compounds may therefore partly explain the toxic effects of T.N.T. The blood of dogs exposed to T.N.T. contains methaemoglobin and this appears to be the only abnormal pigment detectable spectrophotometrically. An insoluble material, which is denatured globin, is also present (Horecker, 1942). Both the dinitroaminotoluenes are relatively nontoxic and are probably true detoxication products of the hydroxylamine and consequently of T.N.T. itself.

The work of Lemberg and Callaghan (1944) suggests that the metabolism of T.N.T. in the rat is slightly different from that in the rabbit. The rat excretes only one dinitrotoluidine, namely, 4-amino-2:6-dinitrotoluene. Lemberg and Callaghan also report the isolation from the urine of T.N.T.-treated rats of a compound which they think is 5-nitro-*m*-phenylenediamine. If this is correct, then it appears that the rat is able to remove the methyl group from T.N.T. The *in vivo* removal of a nuclear methyl group is uncommon, but should the methyl group in T.N.T. be oxidised in the body to carboxyl, then there would be produced 2:4:6-trinitrobenzoic acid which is known to lose $CO_2$ readily; the formation of nitrophenylenediamine is therefore feasible.



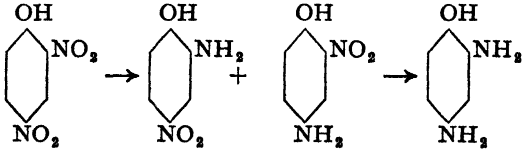
**5-nitro-*m*-phenylenediamine**

In man T.N.T. appears to be converted to the same metabolites as in the rabbit. Dale (1921) showed that 2:2′:6:6′-tetranitro-4:4′-azoxytoluene could be isolated from the urine of T.N.T. workers, a fact which indicates that T.N.T. is reduced in man to 4-hydroxylamino-2:6-dinitrotoluene. Lemberg and Callaghan (1944) also detected the two aminodinitrotoluenes in human urine and they state that the qualitative and quantitative distribution of T.N.T. metabolites in human urine is similar to that found in rabbit urine.

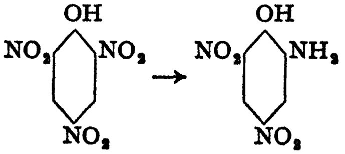
## THE NITROPHENOLS

**The Mononitrophenols.** Since these compounds contain both a nitro and a phenolic hydroxyl group it is to be expected that they will undergo changes *in vivo* which are characteristic of both groups. All three nitrophenols conjugate with sulphuric and glucuronic acid (cf. Meyer, 1905; Baumann and Herter, 1877–8; Williams, 1938) through their hydroxyl groups, but reduction of the nitro group is dependent upon its position in relation to the phenolic group. *m*- and ***p*-Nitrophenol** are partly reduced to *m*- and *p*-aminophenol respectively, but the *ortho* compound is probably only reduced with difficulty, if at all (Meyer, 1905). This is of interest for the least toxic of the three is ***o*-nitrophenol**, the most toxic being the *para*-compound (Gibbs and Hare, 1889; 1894), and it appears probable that their toxicities may be related to the extent and the rate at which they are reduced (presumably through nitrosophenols and hydroxyphenylhydroxylamines to aminophenols).

**Dinitrophenols.** Of the six dinitrophenols only one, **2:4-dinitrophenol,** is of importance. It is used in the explosives industry, and owing to its ability to stimulate cellular respiration by several hundred per cent it has been used as a slimming agent. In the animal body it is excreted partly unchanged, partly conjugated with glucuronic acid through its hydroxyl group and partly reduced to 2-amino-4-nitro-, 2-nitro-4-amino-, and probably 2:4-diaminophenols (Guerbet and Mayer, 1932). The reduced phenols are also excreted combined with glucuronic acid.



**Trinitrophenols.** **Picric acid** (2:4:6-trinitrophenol) is an important substance, but little is known regarding its fate in the animal body. Whereas some is excreted unchanged, it is also partly reduced to picramic acid (4:6-dinitro-2-aminophenol) which is excreted in the urine. Other



picric acid picramic acid

products including a red pigment are also excreted, but their composition is unknown (Karplus, 1893). Karplus found an increased excretion of ethereal sulphates in a case of picric acid poisoning, but Walko (1901) found that picric acid and its metabolite picramic acid did not increase the ethereal sulphate output in rabbits. Walko further showed picramic acid to be more toxic than picric acid.

**The Nitrobenzoic Acids.** The nitrobenzoic acids, particularly the *para* and *meta* isomerides, appear to possess therapeutic activity. ***p*-Nitrobenzoic acid** shows a marked antibacterial action in experimental streptococcal and pneumococcal infections in mice (Mayer and Oechslin, 1939). Against pneumococcus Type II, the bacteriostatic effects of *p*-nitrobenzoic acid and its sodium salt are about equal to that of sulphathiazole (King and Henschel, 1941). Sodium *p*-nitrobenzoate in concentrations up to 0·1% inhibits the growth of a certain strain of *Streptococcus viridans*, but higher concentrations, i.e. up to 0·4%, are without effect. With these higher concentrations the corresponding amino compound becomes detectable in the culture, and no doubt this inhibits the activity of the *p*-nitrobenzoate, for the latter, like sulphanilamide, is inhibited by *p*-aminobenzoic acid (Miller, 1941). Growth in the particular strain of *Streptococcus viridans* used by Miller was not stimulated by *p*-aminobenzoic acid.

**Sodium *m*-nitrobenzoate** has been reported to possess trypanocidal action. Of a number of derivatives of benzoic acid tested against *Trypanosoma equiperdum* infections in mice, sodium *m*-nitrobenzoate was the most efficient, although relapses were common. This activity is lost if the carboxyl group is replaced by OH, Cl or $SO_2NH_2$ (Rosenthal and Bauer, 1941).

There is little information regarding the fate of the nitrobenzoic acids in the animal body, although much can be inferred from the fate of the corresponding nitrobenzaldehydes (see p. 85). It will be recalled that in man the nitrobenzaldehydes are mainly converted to the corresponding nitrobenzoic acids and the nitro group does not appear to undergo reduction, whereas in the rabbit the *m*- and *p*-isomerides, but not the *o*-, are partly reduced to aminobenzoic acids. The early work of Bertagnini (1851) indicated that in man, *p*-nitrobenzoic acid is partly excreted as *p*-nitrohippuric acid. In the dog ***o*-nitrobenzoic acid** is mainly excreted unchanged; the *m*-acid forms appreciable quantities of both a glycine and a glucuronic acid conjugate, whereas the *para* acid forms only small amounts of glycine and glucuronic acid conjugates (Quick, 1932).

In the rat, after ingestion or intraperitoneal injection, 72% of the *ortho*-, 63% of the *meta*- and 89–94% of the *para*- compounds can be accounted for as metabolites in the urine in 48 to 72 hours. All three are reduced; the extent of reduction of the three nitrobenzoic acids is given in Table 18 (Kohl and Flynn, 1941).

*m*- and *p*-Nitrobenzoic acids are also reduced to diazotisable amino substances by suspensions of rat or mouse liver or rat kidney; the *ortho* compound, however, is not reduced by these tissue preparations (Kohl and Flynn, 1941).

The results below indicate that the nitrobenzoic acids which possess chemotherapeutic activity are those which undergo metabolic changes *in vivo*. The *m*- and *p*-isomerides are both partly reduced, they form

TABLE 18

*The Reduction of* o-, m- *and* p-*Nitrobenzoic Acids in the Rat*

Dose 20 mg.

| Nitrobenzoic Acid | Percentage of dose recovered in urine | Amino compound formed as percentage of total recovered | |
|---|---|---|---|
| | | Free | Conjugated |
| *Ortho* | 72 | 21 | not measured |
| *Meta* | 63 | 8 | 22 |
| *Para* | 89–94 | 2·5–4 | 11–20 |

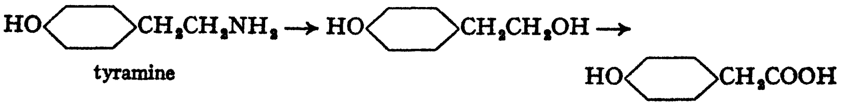
conjugates *in vivo,* and these are the active isomerides. The *ortho* compound, which does not appear to possess chemotherapeutic properties, is probably excreted unchanged by man, the dog and the rabbit, but not by the rat.

## AROMATIC AMINO COMPOUNDS

The aromatic amino group is characterised by its stability in the animal body, and this is in contrast with the aliphatic amino group. The word stability is used in the sense that it is difficult to remove *in vivo* from the aromatic ring and also that the modifications it undergoes are comparatively few.

For purposes of comparison let us consider briefly the fate of the various types of aliphatic amino group.

(*a*) **Aliphatic Primary Amines. Methylamine** is said to be completely metabolised *in vivo* (Kapellar-Adler and Krael, 1931), the amino group probably being split off as ammonia. Pohl (1892) claims that small amounts of formate are excreted by dogs receiving methylamine hydrochloride. Other work has suggested that compounds such as benzylamine, tyramine, *iso*amylamine and indolethylamine are converted with the loss of the amino group, probably *via* the corresponding alcohol, to benzoic, *p*-hydroxyphenylacetic, *iso*valeric and indoleacetic acids, respectively (Guggenheim and Loeffler, 1916; Imai, 1924; Go, 1927) e.g.



However, studies of the action of amine oxidase which is found in various tissues such as liver, kidney and intestine, on various primary aliphatic amines have shown that they are in all cases catalytically oxidised to an aldehyde and ammonia (cf. Green, 1941*a*):—

$$RCH_2\overset{+}{N}H_3 + H_2O + O_2 \rightarrow RCHO + \overset{+}{N}H_4 + H_2O_2$$

According to Richter (1937) the reaction may be split into two stages, in which the amine is first dehydrogenated to the corresponding imine, which reacts spontaneously with water forming an aldehyde and ammonia :—

$$\begin{array}{l}\mathrm{RCH_2NH_2 + O_2 \longrightarrow RCH{=}NH + H_2O_2}\\ \mathrm{RCH{=}NH + H_2O \longrightarrow RCHO + NH_3}\end{array}$$

Aniline is not affected by amine oxidase.

(*b*) **The Aliphatic Secondary Amines. Diethylamine** is said to be destroyed *in vivo*, none of it being excreted unchanged (Hildebrandt, 1901). Amine oxidase catalytically oxidises aliphatic secondary amines to an aldehyde and a lower amine (cf. Green, 1941*a*) :—

$$\mathrm{RCH_2\overset{+}{N}H_2R_1 + H_2O + O_2 \longrightarrow RCHO + \overset{+}{N}H_3R_1 + H_2O_2}$$

In this case there are two alternative paths for enzymic oxidation, as follows :—

$$\mathrm{RCH_2NHCH_2R_1}\begin{array}{l}\nearrow \mathrm{RCHO + NH_2CH_2R_1}\\ \searrow \mathrm{R_1CHO + NH_2CH_2R}\end{array}$$

(*c*) **Aliphatic Tertiary Amines. Trimethylamine** given orally to dogs is converted mainly to ammonia, which corresponds to about 50% of the dose, whereas the rest is excreted as trimethylamine oxide, unchanged trimethylamine and traces of what is probably dimethylamine (Müller, 1940).

$$\mathrm{(CH_3)_3N \longrightarrow NH_3 + (CH_3)_3NO + ?(CH_3)_2NH}$$

Tertiary amines are also oxidised catalytically by amine oxidase and the general reaction can be represented as follows (cf. Green, 1941*a*) :—

$$\mathrm{RCH_2\overset{+}{N}H(R')_2 + H_2O + O_2 \longrightarrow RCHO + \overset{+}{N}H_2(R')_2}$$

(*d*) **The Amino Acid NH$_2$ Group.** In the α-position the amino group is removed by the well-known mechanism of α-oxidative deamination and eventually converted mainly to urea. Deamination, however, is not confined to the α-position,[1] for Mayer (1904) has shown that with **α:β-diaminopropionic acid** both amino groups are removed, yielding α:β-dihydroxypropionic acid. The NH$_2$ group of amino acids may also be acetylated *in vivo* and Knoop has suggested that the acetylation of amino acids may be concerned in their synthesis. Thus Knoop and Kertess (1911) (cf. Fishman and Cohn (1943) and Bernhard and Steinhauser (1942) ) found that on feeding 18 g. of *dl*-γ-phenyl-α-aminobutyric acid to dogs, 4 g. of *d*-γ-phenyl-α-acetaminobutyric acid could be isolated from the urine ; small amounts of the acetylated amino acid were also formed when γ-phenyl-α-keto- and γ-phenyl-α-hydroxybutyric acids were fed. Many examples of this reaction are known. We see, therefore,

[1] It is interesting to note that δ-aminovaleric acid forms 4-amino-2-butanone when fed to dogs (Keil, 1927) :—

$$\mathrm{NH_2(CH_2)_4COOH \longrightarrow NH_2CH_2CH_2COCH_3}$$

that in most cases the aliphatic amino group can be split off as ammonia, although in some cases it can also undergo acetylation.

The aromatic amino group is not known to be split off. The only established biological change which it undergoes is acetylation and even this is said to be non-enzymic. That it can be oxidised to a hydroxylamine is unlikely, although opinions are divided on this point (cf. Thorpe and Williams, 1941 ; Rimington, 1939 ; Rimington and Hemmings, 1939 ; Heubner and Schwedtke, 1936). When the aromatic amino group occurs in conjunction with an *ortho* or a *para* hydroxyl group, it can, in solution, set up a redox system with the corresponding quinoneimine. There is little doubt that this system occurs *in vivo*, but the change from $—NH_2$ to $=NH$ is here purely chemical and not biochemical. The change is, however, of great importance when considered in relation to the toxic effects produced by aromatic amino compounds.

There is, therefore, a sharp contrast between the behaviour of aromatic and aliphatic amino groups *in vivo*, the former being stable and the latter easily split off as ammonia. This difference may be related to the fact that the aliphatic amino group occurs in many normal body constituents, whereas very few such compounds contain the aromatic amino group.

**The Acetylation of Amino Compounds *in vivo*.** When considering the mechanism of the biological acetylation of amino groups, it is important to remember that at least two types of molecules undergo this process, namely, those with an α-amino acid $NH_2$ and those with an aromatic $NH_2$. All animals (see p. 161) except the dog can acetylate the aromatic $NH_2$ ; the dog, like other animals, can, however, acetylate the amino acid $NH_2$. This peculiarity of the dog suggests that the acetylation of the aromatic $NH_2$ is a process differing from that of the amino acid $NH_2$, but there is, as yet, no evidence to decide this point.

It seems certain that the main site of acetylation is the liver[1] (Klein and Harris, 1938), but the nature of the immediate acetylating agent is not clear, although there is much evidence to show that acetate can provide the necessary carbon residue. Working with rabbit liver slices, Klein and Harris showed that lactate and pyruvate could provide acetate for the acetylation of sulphanilamide in the following ways :—

i. lactate $\xrightarrow{a}$ pyruvate $\xrightarrow{b}$ acetate + sulphanilamide $\xrightarrow{c}$ acetylsulphanilamide

ii. pyruvate $\longrightarrow$ acetaldehyde $\longrightarrow$ acetate + sulphanilamide $\xrightarrow{c}$ acetylsulphanilamide

The process (*c*) is irreversible, non-enzymic[2] and varies greatly in animals of the same species. Direct proof of the rôle of acetate in the acetylation of amino groups was first provided by Bernhard (1940) who showed that when foreign amines were fed with deuteroacetic acid to dogs acetylated amines were excreted containing deuteroacetyl groups.

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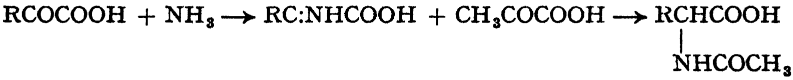
[1] The work of Maher (1944) indicates that in the rabbit the reticulo-endothelial system may be concerned in the acetylation of sulphonamides.

[2] See footnote p. 141.

This has been confirmed by Bloch and Rittenberg (1944). The use of deuteroethyl alcohol has shown that ethanol is as efficient as acetate in supplying the acetyl group (Bernhard; Bloch and Rittenberg). Acetate is produced in large amounts in tissues during normal metabolic processes (Bloch and Rittenberg, 1944).

When rats are given water containing deuterium oxide to drink and are then fed *p*-aminobenzoic acid, sulphanilamide or $\gamma$-phenyl-$\alpha$-aminobutyric acid, the acetyl groups of the excreted acetyl derivatives of these three compounds contain approximately the same amount of deuterium (Fishman and Cohn, 1943). Here the deuterium in the acetyl groups must be derived from body water. Deuterium from body water does not exchange with preformed acetyl groups, for when the preformed acetylated amines are fed with deuterium oxide, the excreted compounds do not contain deuterium in their acetyl groups. Deuterium from body water must therefore be taken up by the precursor of the acetyl group before or during acetylation. Fishman and Cohn therefore postulate that the precursor of the acetyl group must be a molecule which is capable of exchanging its hydrogen or reacting chemically with water. This substance cannot be pyruvic acid for the latter does not undergo exchange reactions in deuterium oxide solutions. Exchange of deuterium and hydrogen might be possible during the synthesis of acetylated amino acids according to Knoop's scheme involving ammonia, pyruvic acid and the ketonic acid:—



$$RCOCOOH + NH_3 \longrightarrow RC{:}NHCOOH + CH_3COCOOH \longrightarrow \underset{\displaystyle NHCOCH_3}{\underset{|}{RCHCOOH}}$$

But this is not possible during the acetylation of an aromatic amine, and in any case phenylaminobutyric acid and aromatic amines when acetylated *in vivo* in the presence of $D_2O$ contain the same amount of deuterium, a fact which seems to indicate a common acetylating agent for both the aromatic $NH_2$ and the amino acid $NH_2$. The work of Fishman and Cohn suggests that neither acetate nor pyruvate can be the direct acetylating agent. Doisy and Westerfeld (1943) have shown that acetoin is a possible precursor of the acetylating agent and that acetoin can be formed from pyruvate and acetaldehyde.

The present position is that acetate is involved in the acetylation of the amino group, but whether or not it is the direct acetylating agent[1] *in vivo* has yet to be decided.

**Aniline.** The main product found in the urine as a result of the metabolism of aniline is *p*-aminophenol (Schmiedeberg, 1878). This compound occurs in the urine not only in the free state but also as the acetylated ethereal sulphate and probably the glucuronide, for Mörner

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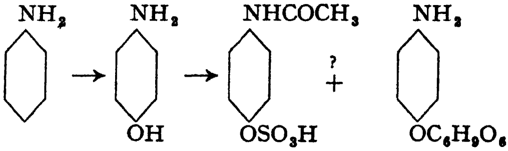
[1] The recent work of Lipmann (1945) indicates that the acetylation of sulphanilamide is an enzymic process involving the participation of acetate and adenylpyrophosphate. Lipmann believes that acetate and sulphanilamide are enzymically induced to form a complex with adenylpyrophosphate which exergonically breaks up to acetylsulphanilamide, adenylic acid and inorganic phosphate.

(1889) has isolated potassium *p*-acetaminophenyl sulphate from the urine of patients receiving acetanilide (acetanilide is hydrolysed to aniline *in vivo*) and Williams (1943) has isolated *p*-aminophenylglucuronide from the urine of rabbits receiving *p*-aminophenol.



In rats, aniline and *p*-aminophenol are about equally toxic, for the L.D.50 of aniline is 460 mg./kg. and of *p*-aminophenol is 465 mg./kg. (Vandenbelt, Pfeiffer, Kaiser and Sibert, 1944).

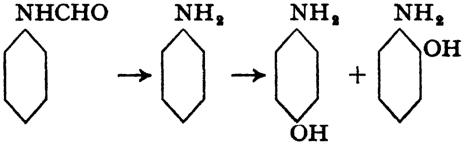
*o*-Aminophenol is also a possible product, but its formation has not been proved. According to Zehender (1943) about 41% of a 0·1 g./kg. dose of aniline hydrochloride is excreted by guinea-pigs in 8 hours in a form containing a diazotisable amino group, and one-fifth of this is in an acetylated form. The formation of *p*-aminophenol from aniline may take one of two paths :—(*a*) direct oxidation of the ring or (*b*) oxidation of the amino group to phenylhydroxylamine which then rearranges to *p*-aminophenol. Of these two alternatives, the former appears to be more feasible, for direct oxidation of the benzene ring is a well-known biological reaction (the exact mechanism of the oxidation of aromatic rings is unknown). When a hydroxyl group, for example, is already present in the ring, the oxidation always takes place *ortho* or *para* to it if these positions are free. Furthermore, in amino compounds where hydroxylamine formation is excluded by substitution, oxidation to the phenol takes place *para* to the substituted amino group, for Horn (1937) showed that in the dog and the rabbit diethylaniline is oxidised to *p*-diethylaminophenol.

The intermediate formation of phenylhydroxylamine was supported by Ellinger (1920), who claimed to have isolated in minute amounts acetylphenylhydroxylamine from the blood of cats poisoned by acetanilide. Ellinger's product, as pointed out by Thorpe and Williams (1941), was never definitely proved to be a hydroxylamine. There is little doubt that the aromatic nitro group can be reduced to a hydroxylamine *in vivo*, but it is unlikely that the amino group can be oxidised to a hydroxylamino group. In any case oxidation of the aromatic amino group is a difficult process even chemically. The results of Böhm (see p. 225) on the formation of indican *in vivo* can also be interpreted as being against the theory that aniline, or even any other aromatic amino compound, can be oxidised to phenylhydroxylamine. Böhm showed that whereas *o*-nitropropiolic acid was converted to indican in the animal the corresponding aminopropiolic acid was not. This worker claimed that the *o*-nitro group was converted to a hydroxylamino group which then reacted to form a ring with the *ortho* side-chain. If the hydroxylamino group were a common metabolite of both the nitro and the amino group, then one could have expected both *o*-nitro and *o*-aminopropiolic acid to form indican.

Recent results obtained by Lester, Greenberg and Shukovsky (1944; see also Lolli, Lester and Rubin, 1944) on the extent of methaemoglobin formation in rats receiving aniline, *β*-phenylhydroxylamine and *p*-aminophenol indicate that *p*-aminophenol is not the only methaemoglobin-former produced during the metabolism of aniline. It was found that the maximum extent of methaemoglobin formation from *p*-aminophenol was 30–35% of the blood pigment at a minimum dose of 320 mg./kg. (i.e. half the toxic dose); for *β*-phenylhydroxylamine, the maximum methaemoglobin formation was 63–66% with a minimum dose of 5 mg./kg. (one-seventh the toxic dose), whereas for aniline hydrochloride the values were methaemoglobin, 47%, for a minimum dose of 300 mg./kg. The maximum methaemoglobin formation with aniline hydrochloride is above that which can be obtained with *p*-aminophenol, thus suggesting that if the latter is a product of the metabolism of aniline it is not the only methaemoglobin-forming metabolite. The nature of the other metabolites was not defined by Lester *et al.* Other possible metabolites are *o*-aminophenol and *β*-phenylhydroxylamine.

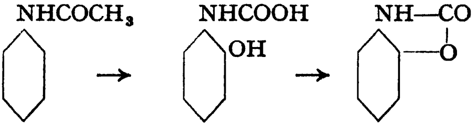
## N-SUBSTITUTED DERIVATIVES OF ANILINE

**Formanilide.** Kleine (1896–7) found that formanilide was excreted by dogs as *p*-aminophenol; benzoxazolone was also isolated. In other words, the formyl group is removed and the resulting aniline is oxidised in both the *o*- and *p*-positions.



The mechanism of the formation of benzoxazolone is discussed below. In rabbits the main product is *p*-aminophenol, excreted both as glucuronide and as ethereal sulphate.

**Acetanilide.** The first stage in the metabolism of acetanilide is probably deacetylation to aniline, for Michel, Bernheim and Bernheim (1937) have shown that both rat liver and kidney contain an acylase which is capable of deacetylating acetanilide. The metabolism of acetanilide is dependent to some extent upon the species of animal studied. In humans it is converted mainly to *p*-aminophenol, which is excreted mainly as *p*-acetylaminophenylsulphate and *p*-aminophenylglucuronide (cf. aniline) (Müller, 1887; Mörner, 1889). In the dog acetanilide is mainly oxidised to *o*-aminophenol which is obtained from the urine as benzoxazolone (oxycarbanil), together with small amounts of *p*-aminophenol. The formation of benzoxazolone requires some discussion. It has been obtained from the urine of dogs, which have received formanilide or acetanilide (see also *o*-acetotoluidide), after evaporation of the urine to dryness in the presence of concentrated HCl. It is well known that chemically *o*-aminophenol derivatives are easily converted to benzoxazole derivatives. It appears likely that the compound is an artefact[1] and the true metabolic product is *o*-aminophenol. Its formation, however, was considered to take place *in vivo* by Jaffe and Hilbert (1888), who represented the change as follows:—



In rabbits acetanilide is deacetylated and converted into *p*-aminophenol which is excreted as an ethereal sulphate and a glucuronide. It appears probable that in both animals the fate of acetanilide can be represented as follows:—

acetanilide ⟶ aniline ⟶ *o*- and *p*-aminophenol ⟶ ethereal sulphates and glucuronides.

The difference between the dog and rabbit lies in the proportion of each phenol formed, for in the dog the *o*-derivative is the main product, whilst in the rabbit the *p*-phenol is predominant. This species difference also occurs with dimethylaniline (see below).

## N-ALKYLANILINES

The fate of N-**monomethylaniline** in the animal body does not seem to have been studied other than to show that it is a methaemoglobin-former.

N:N-**Dimethylaniline** was first investigated by Hildebrandt (1907 who claimed that in rabbits it was first oxidised to dimethylaniline oxide, which then rearranged to *p*-dimethylaminophenol.

$$C_6H_5N(CH_3)_2 \longrightarrow C_6H_5N(CH_3)_2{:}O \longrightarrow (CH_3)_2NC_6H_4OH$$

The question of the intermediate formation of the amine oxide has been fully investigated by Horn (1936), who found a striking difference between the metabolism of dimethylaniline in the rabbit and in the dog. In the former animal, the compound on subcutaneous injections is

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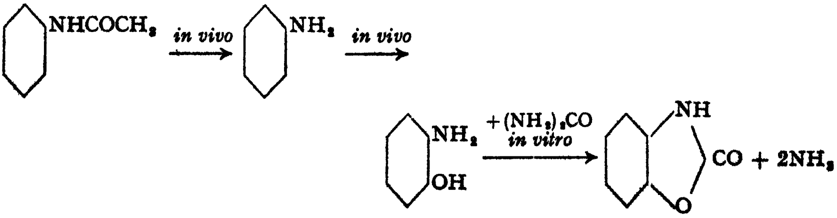
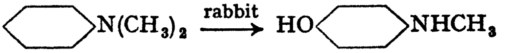
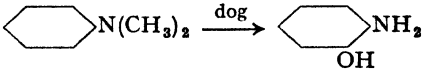
[1] Recent work (unpublished) by the writer has shown that *o*-aminophenol condenses readily with urea at 160° C. with the elimination of ammonia to form benzoxazolone. The formation of benzoxazolone from acetanilide probably proceeds as follows:—



partly demethylated and oxidised in the *para* position and there is excreted in the urine the ethereal sulphate and glucuronide of monomethylaminophenol :—

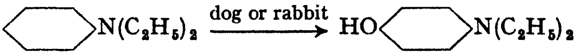


In the dog, on the other hand, dimethylaniline is completely demethylated and oxidised in the *ortho* position, and there is excreted the ethereal sulphate and glucuronide of *o*-aminophenol. Horn does not record the isolation of benzoxazolone (cf. acetanilide), as would be expected if the compound were not an artefact.



In neither case was dimethylaniline oxide excreted, and when this compound was injected it was converted into the same products as dimethylaniline.

N:N-**Diethylaniline.** The metabolism of diethylaniline differs markedly from that of dimethylaniline, in that it is not de-ethylated in either the dog or the rabbit, and in both animals oxidation is *para* to the diethylamino group (Horn, 1937), no difference being observed between the herbivorous and carnivorous animals. The *p*-diethylaminophenol formed in each case is excreted conjugated.



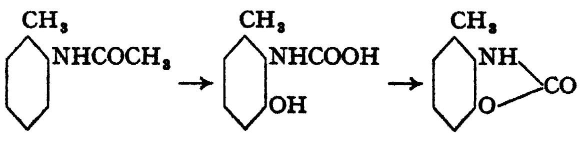
**Diethylaniline oxide** is not an intermediate in this reaction, for when injected into either animal it is partly excreted as *p*-diethylaminophenol but chiefly unchanged.

## TOLUIDINES AND THEIR DERIVATIVES

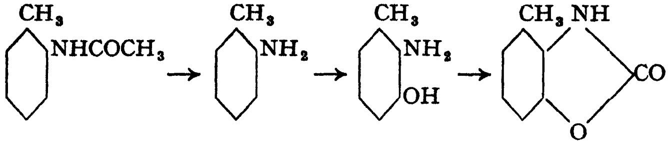
Little is known regarding the fate of the toluidines as such. *o*-**Toluidine** is excreted as an ethereal sulphate and is apparently oxidised in dogs mainly to an aminophenol, the identity of which is so far obscure (Hammerbacher, 1883–4 ; Kuchenbecker, 1920). *p*-**Toluidine**, on the other hand, does not form an ethereal sulphate and is probably oxidised partly to *p*-aminobenzoic acid (Hildebrandt, 1907).

Regarding the acetotoluidides, we have much more information (Jaffe and Hilbert, 1888), and since it is likely that these compounds are first deacetylated *in vivo* to the corresponding toluidines, conclusions regarding the fate of the latter compounds can be made.

*o*-**Acetotoluidide**, according to Jaffe and Hilbert, is excreted in the dog mainly as 4-methyl-2-oxybenzoxazole, and they give the mechanism of its formation as follows :—

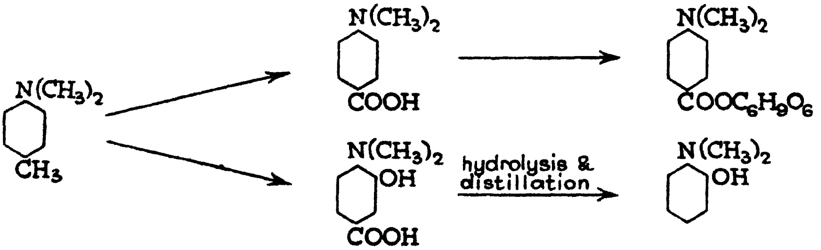


This is probably incorrect since the benzoxazolone derivative is likely to be an artefact. A more probable mechanism is that acetotoluidide is first deacetylated to *o*-toluidine, which is then oxidised to 2-amino-3-methylphenol, which is then probably converted to methylbenzoxazolone as a result of the isolation procedures thus :—

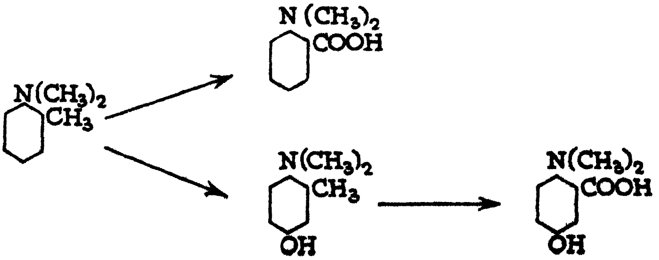


The *m*- and ***p*-acetotoluidides** are excreted by the rabbit and by the dog as the corresponding *m*- and *p*-acetaminobenzoic acids (Jaffe and Hilbert, 1888). It is known that the dog cannot acetylate the aromatic amino group like other animals, and in this case it appears as if the original acetyl groups of the acetotoluidides fed, have survived unchanged.

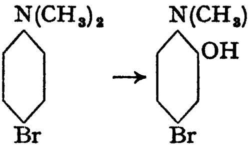
***p*-N-Dimethylaminotoluene** when fed to rabbits results in the excretion of a reducing urine. The compound responsible is probably *p*-N-dimethylaminobenzoylglucuronide (see p. 86), for *p*-N-dimethylaminobenzoic acid can be isolated from the urine. If the urine is hydrolysed with acid and then distilled, *o*-N-dimethylaminophenol is produced which is probably derived from *p*-N-dimethylamino-*m*-hydroxybenzoic acid (Hildebrandt, 1906; 1907) :—



***o*-N-Dimethylaminotoluene** undergoes in the rabbit a similar series of changes forming *o*-dimethylaminobenzoic acid and probably 3-hydroxy-6-N-dimethylaminobenzoic acid. The latter compound is not an oxidation product of *o*-dimethylaminobenzoic acid, for when this acid is fed to rabbits, the phenol is not produced (Hildebrandt, 1907). The formation of the phenol is probably an independent reaction with 6-dimethylamino-3-hydroxytoluene as a possible intermediate :—

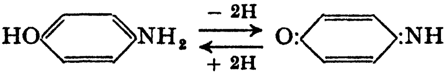


Hildebrandt also found that *p*-bromo-N-dimethylaniline undergoes a similar oxidation in rabbits forming *p*-bromo-*o*-hydroxy-N-dimethylaniline thus :—



## METHAEMOGLOBIN FORMATION AND THE METABOLISM OF AROMATIC AMINO COMPOUNDS

One of the most interesting effects of aromatic amino compounds in animals is their action on the blood pigment and the formation of methaemoglobin, and it appears that this effect is closely related to their metabolism. In the metabolism of amino compounds the most frequent products are *o*- and *p*-aminophenols. These compounds are formed during the metabolism of nitro compounds, but here, in addition, hydroxylamines,[1] which are powerful methaemoglobin-formers (Heubner, Meier and Rhode, 1923), may also be produced. Nitroso compounds are also possible metabolites of nitro compounds and are methaemoglobin-formers, e.g. Heubner and Meier (1923) have shown that nitrosobenzene converts oxyhaemoglobin into equal amounts of methaemoglobin and reduced haemoglobin. It is well known that *o*- and *p*-aminophenols can set up in solution redox systems consisting of the aminophenol and its oxidation product, quinoneimine, e.g.



According to Heubner (1913) (see also Heubner and Meier, 1923) the production of *o*- or *p*-aminophenols *in vivo* can explain the formation of methaemoglobin according to the following equations :—

$$o\text{- or } p\text{-}HOC_6H_4NH_2 + O \longrightarrow O{:}C_6H_4{:}NH + H_2O$$

$$O{:}C_6H_4{:}NH + \underset{\text{haemoglobin}}{2(Hb{:}Fe)} + 2H_2O \longrightarrow HOC_6H_4NH_2 + \underset{\text{methaemoglobin}}{2(Hb{:}FeOH)}$$

According to the above equations, one molecule of aminophenol can oxidise two atoms of haemoglobin iron, but since quinoneimine is regenerated, one molecule of aminophenol can convert an almost unlimited quantity of haemoglobin to methaemoglobin. According to Rimington and Hemmings (1939), the chemical grouping necessary for methaemoglobin formation is an aromatic amino group, unsubstituted or potentially free and preferably in such a system that it is capable of undergoing oxidation with the formation of a hydroxylamine derivative or a reversibly oxidising system such as a *p*- and *o*-quinoneimine. Rimington and Hemmings also point out that substances which produce methaemoglobin also cause porphyrinuria. A few examples (see, for example, Rimington and Hemmings, 1939) will illustrate the above generalisations; aniline, acetanilide, *o*-toluidine, dimethylaniline are methaemoglobin-formers and, as can be seen from earlier paragraphs, they are oxidised *in vivo* to aminophenols. The *m*- and *p*-acetotoluidides are not methaemoglobin-formers (Ellinger, 1920) for they are both oxidised to the corresponding acetaminobenzoic acids (Jaffe and Hilbert, 1888). As pointed out by Rimington and Hemmings, this property of catalysing methaemoglobin formation need not be confined to the system aminophenol $\rightleftarrows$ quinoneimine, since it is possible for compounds containing fully substituted amino groups to set up such systems, for compounds of this type are capable of stepwise oxidation to semiquinones. Thus diethylaniline which is not de-ethylated *in vivo*, is oxidised *in vivo* to diethylaminophenol, and is at the same time a methaemoglobin-former (Horn).

---

[1] *In vitro* phenylhydroxylamine converts oxyhaemoglobin to methaemoglobin and is itself changed to azoxybenzene. When phenylhydroxylamine is present in excess, the methaemoglobin formed in the primary reaction is reduced to haemoglobin by the excess phenylhydroxylamine, and if this is not too great a third change occurs and more methaemoglobin is formed but in much less amount than in the primary reaction. Phenylhydroxylamine does not react with reduced haemoglobin in the absence of air (Heubner *et al.*, 1923).

Sulphaemoglobin frequently accompanies methaemoglobin when aromatic amino compounds are fed to animals. Thus Morgan and Anderson (1940) found sulphaemoglobin in the blood of a woman suffering from chronic acetanilide poisoning, whilst Smith (1940) found twice as much sulphaemoglobin as methaemoglobin in the blood of rats which had received orally sulphanilamide daily for three months. The possibility that *p*-aminophenol is concerned with the formation of sulphaemoglobin is suggested by Morgan and Anderson (1940) and Campbell and Morgan (1939), who showed that when *p*-aminophenol is added to laked blood containing traces of sulphide, sulphaemoglobin is rapidly formed.

Rabbits are particularly resistant to methaemoglobin formation whereas the cat is particularly sensitive. Lester (1943) has investigated this further and found with acetanilide and phenacetin that the rabbit and monkey form virtually no methaemoglobin. The cat is the most sensitive to methaemoglobin formation by these drugs, whereas man is half as sensitive as the cat, the dog half as sensitive as man and the rat one-sixth as sensitive as the dog. These differences may be taken to indicate possible differences in the metabolism of these drugs in various animals, although species differences in the haemoglobin must not be entirely overlooked.

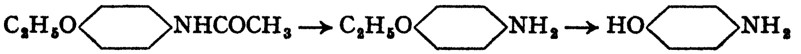
Recent views (Lester *et al.*, 1944; Lolli *et al.*, 1944) suggest that methaemoglobin formation plays little part in the acute toxicity of aniline, acetanilide, *p*-aminophenol and related compounds, for maximum formation of methaemoglobin from β-phenylhydroxylamine and *p*-aminophenol (see p. 142) occurs at doses of these drugs which produce no appreciable toxic effect or evident symptoms of anoxaemia. Furthermore, on increasing the dose of these compounds, no further formation of methaemoglobin occurs, although symptoms of drug intoxication (and finally death) appear. The maximum amount of methaemoglobin formed, however, varies with the drug and the species of animal. Death only occurs, in dogs, at 95% methaemoglobinaemia and recovery may occur at 87%; such values have been obtained with *p*-aminopropiophenone and *p*-aminoacetophenone (Vandenbelt *et al.*, 1944).

*o*-**Phenylenediamine** and *m*-**phenylenediamine** have not been studied as regards their fate in the body. They are, however, likely to differ from the *p*-isomeride since they do not form a blue pigment in frog muscle as does *p*-phenylenediamine (Matsumoto, 1901). *p*-**Phenylenediamine** *in vivo* is converted in the first place to quinonediimine which readily polymerises to a black pigment (Erdmann and Vahlen, 1906) and its methaemoglobinaemic action is very likely to be due to this diimine (Heubner, 1913). It seems probable that both amino groups can be acetylated *in vivo* (see p. 152).

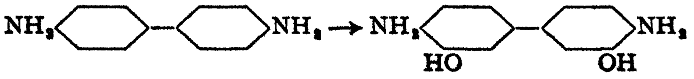
**The Aminophenols.** The fate of these phenols in the animal body seems fairly clear. All three are excreted as ethereal sulphates and glucuronides. In the rabbit at a dose level of 290 mg./kg. they form ethereal sulphates to the extent of 26–29% for the *ortho* compound, 31–36% for the *meta* and 20% for the *para*, whereas an equivalent dose of phenol is conjugated with sulphate to the extent of 20% (Williams, 1938). The three glucuronides have been isolated by Williams (1943); both the *o*- and *m*-compounds form considerable amounts of glucuronides in the rabbit and at least 25% of the dose can be obtained as crystalline glucuronides. The *para* compound is conjugated with glucuronic acid to a lesser extent than its isomerides and a moderate amount is excreted in the free state. It appears probable that the *o*- and *m*-aminophenols are excreted mainly in the conjugated form, whereas much of the *p*-compound is excreted unchanged. The glucuronides are laevorotatory; *o*-, $[\alpha]_D - 76°$; *m*-, $[\alpha]_D - 94°$; *p*-, $[\alpha]_D - 83°$.

It has recently been shown that *o*-aminophenol possesses bacteriostatic activity against gram-negative bacilli, which suggests it may be of value as a local antiseptic (Barber and Haslewood, 1944).

One of the most important derivatives of *p*-aminophenol is the antipyretic drug **phenacetin.** In the animal body it is apparently first deacetylated to phenetidine, which is then de-ethylated to *p*-aminophenol, the latter being excreted partly combined with sulphuric and glucuronic acids (Müller, 1888; Mahnert, 1888; Hinsberg and Kast, 1887).



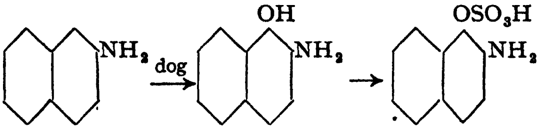
**Benzidine.** This substance is partly excreted unchanged and partly as an ethereal sulphate and a glucuronide (Weber and Heidepriam, 1928). The excretion of these conjugates indicates that it is oxidised *in vivo*, and according to Adler (1907) it forms a diaminodihydroxydiphenyl of unknown structure. Since the *para* positions are blocked it is likely that this compound is 3:3′-dihydroxy-4:4′-diaminodiphenyl.



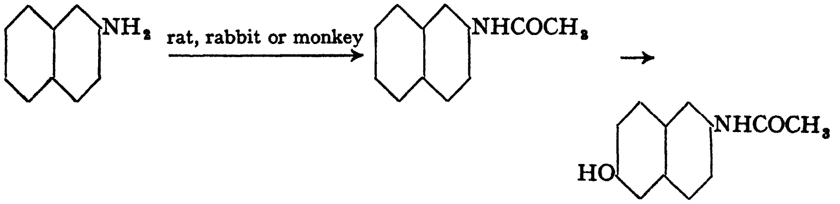
## THE NAPHTHYLAMINES

α-**Naphthylamine** is said to be partly excreted unchanged and partly as α-aminonaphthol which is mainly conjugated with glucuronic acid, and to a small extent as an ethereal sulphate.

β-**Naphthylamine** has been more thoroughly investigated than its isomeride because it is liable to give rise to bladder tumours in workers engaged in its manufacture. Wiley (1938) showed that it was oxidised in the dog to 2-amino-α-naphthol which he isolated as the acid sulphate. As much as 52% of the dose was excreted as ethereal sulphate.



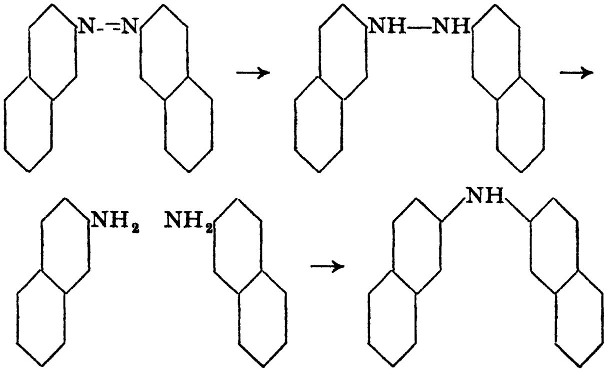
In addition to this there was a rise in neutral sulphur excretion, which indicates that it may form a mercapturic acid. There is a possibility that it might form the carcinogen, 3:4:5:6-dibenzcarbazole (see p. 57). In rats, rabbits and monkeys Dobriner, Hofmann and Rhoads (1941) found that, on subcutaneous injection, it is partly excreted unchanged, partly oxidised and partly acetylated, and from the urine they isolated 2-acetaminonaphthalene and 2-acetamino-6-hydroxynaphthalene, the latter compound probably also forming an ethereal sulphate and a glucuronide.



There is here another illustration of the differences between herbivorous and carnivorous animals, the dog oxidising β-naphthylamine in the *ortho* position, whereas the rabbit oxidises it mainly in a position which is equivalent to *para*. An omnivorous animal like the rat apparently favours *para*-oxidation. The peculiarity of the dog in being unable to acetylate the aromatic amino group is also illustrated once again.

## AZO COMPOUNDS

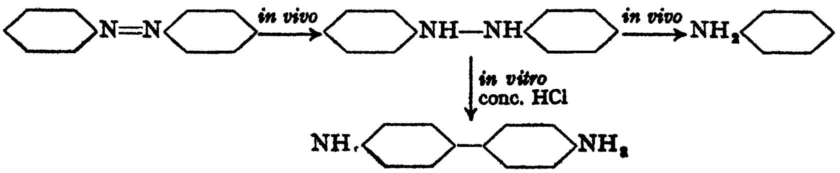
A knowledge of the metabolism of azo compounds in the animal body is of interest for two reasons. In the first place, it was through realising that the azo dye, prontosil, broke down *in vivo* to sulphanilamide, that the present range of sulphonamides for the treatment of acute bacterial infections became available. In the second place, it has been known for a considerable time that certain azo compounds are capable of inducing tumour-like proliferation of tissues. Thus 4′-amino-2:3′-azotoluene and *p*-dimethylaminoazobenzene are active in producing liver tumours in rats and 2:3'-azotoluene induces bladder tumours in the same animal. It has been suggested that the tumorigenic properties of the azo compounds are a result of the changes which they undergo in the body. Cook, Hewett, Kennaway and Kennaway (1940), for example, explain the carcinogenic action of 2:2'-azonaphthalene by suggesting that it may be metabolised to 3:4:5:6-dibenzcarbazole, a known carcinogen of high potency. The mechanism which they suggest for this transformation is that 2:2'-azonaphthalene is first reduced to 2:2'-hydrazonaphthalene, which then undergoes a " benzidine-like " rearrangement to 2:2'-diamino-1:1'-dinaphthyl. The latter subsequently forms 3:4:5:6-dibenzcarbazole, the active carcinogenic agent :—



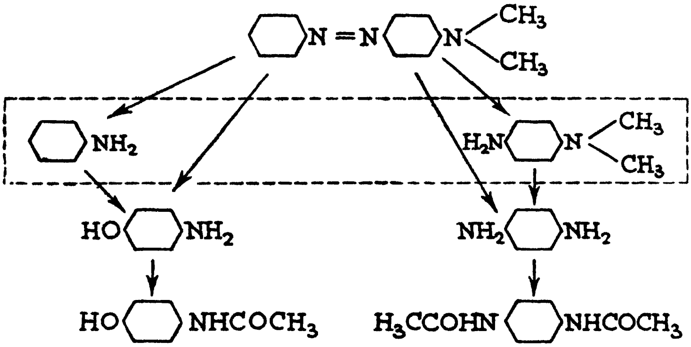
On the evidence available it appears that the azo link is split *in vivo*, both nitrogen atoms being reduced, producing two aromatic amino groups. The hydrazo compound is probably an intermediate in this change. The amines produced are then metabolised, undergoing further changes such as oxidation and acetylation

$$ArN{=}NAr' \longrightarrow ArNH.NHAr' \longrightarrow ArNH_2 + Ar'NH_2$$

**Azobenzene.** Azobenzene does not appear to be carcinogenic. When it had been injected into rats, the urine contained aniline, increased amounts of ethereal sulphates and glucuronides, and a compound which when heated with strong hydrochloric acid yielded benzidine (Elson and Warren, 1944). The precursor of benzidine is without doubt hydrazobenzene, which is probably the primary metabolite of azobenzene. The ethereal sulphate and conjugated glucuronic acid are probably the conjugation products of the further oxidation of aniline (such as *p*-aminophenol) and possibly phenols of hydrazobenzene. The metabolism of azobenzene can be represented as follows :—



N:N-**Dimethyl-*p*-aminoazobenzene** is commonly known as " butter yellow " and it has been shown to possess carcinogenic properties and produces hepatomas in rats. In the rat the azo group is split and both nitrogen atoms reduced ; the methyl groups are also removed. Stevenson, Dobriner and Rhoads (1942) isolated from the urine of rats receiving " butter yellow ", *p*-acetaminophenol, *p*-aminophenol, N:N′-diacetyl-*p*-phenylenediamine, *p*-phenylenediamine and another metabolite of unknown composition. Its fate has been represented by Dobriner *et al.* (1942) as follows :—

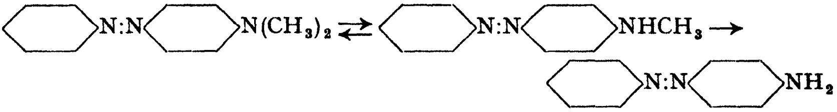


(The compounds within the dotted lines are possible intermediates which were not isolated.)

It should be noted that the above scheme also gives some insight into the metabolism of *p*-phenylenediamine (see p. 149).

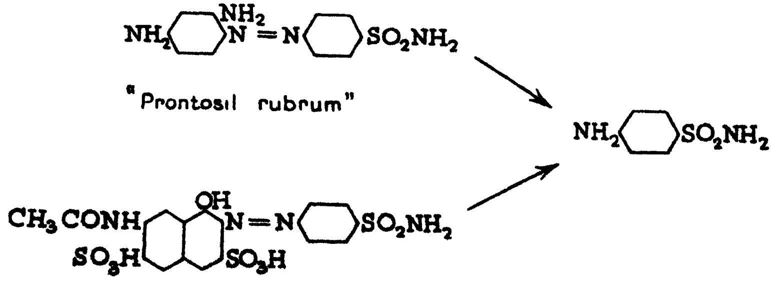
It has been suggested by Rhoads (1942 ; see also Kensler, Dexter and Rhoads, 1942) that " butter yellow " or its metabolites may produce liver cancer by interfering with certain enzyme systems of the liver. Rats are more susceptible to the carcinogenic action of " butter yellow " if they are kept on a riboflavin-deficient diet, and this suggests that the vitamin interacts in some way with the metabolite which causes cancer and perhaps has a rôle in its detoxication. The exact relationship between riboflavin and the carcinogenicity of " butter yellow " has yet to be established. " Butter yellow " also interferes with the activity of coenzyme I (diphosphopyridine nucleotide) which contains the vitamin nicotinic acid, for in rats it produces a " conditioned deficiency " of nicotinic acid, due not to dietary lack of the vitamin but to some interference with the function of the vitamin in the form in which it is biologically active. " Butter yellow " does not inhibit the activity of coenzyme I *in vitro* nor do three of its metabolites, i.e. *p*-aminophenol, *p*-acetaminophenol and diacetyl-*p*-phenylenediamine. The fourth metabolite, *p*-phenylenediamine, however, inhibits the coenzyme I system, and the hypothetical metabolite, dimethyl-*p*-phenylenediamine, is even more powerful in this respect than free diamine. It is suggested, therefore, that " butter yellow " may cause cancer by poisoning, through certain of its metabolites, at least one normal enzyme system thus causing a normal cell to become a cancer cell. The enzymes of a cancer cell can operate apparently in the presence of a compound which, in the same concentration, is poisonous to the enzymes of a normal cell.

However, the recent work of Miller, Miller and Baumann (1945) indicates that N:N-dimethyl-*p*-phenylenediamine is not a metabolite of " butter yellow ". It appears from their results that " butter yellow " is demethylated before the azo link is reduced and split. " Butter yellow " is apparently reversibly demethylated to *p*-monomethylaminoazobenzene which is further irreversibly demethylated to *p*-aminoazobenzene. The feeding of *p*-monomethylaminoazobenzene to rats results in the appearance in the tissues of *p*-dimethylamino- and *p*-aminoazobenzene, as well as the monomethyl derivative itself. The feeding of *p*-aminoazobenzene does not result in the production of methylated derivatives. The first stages in the metabolism of " butter yellow " according to Miller *et al.* are as follows :—

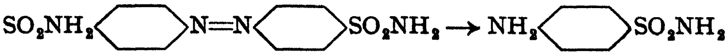


The metabolism of *p*-monomethylaminoazobenzene is of further interest since it provides a new example of biological methylation. Whereas the aromatic —$NH_2$ group is not methylated *in vivo*, the aromatic —$NHCH_3$ group in this particular case is methylated. Whether or not this is a general biochemical reaction for aromatic methylamino groups is a problem for future research.

**The Prontosils.** *In vivo* both "**prontosil rubrum**" (4′-sulphonamido-2:4-diaminoazobenzene hydrochloride) and "**soluble prontosil**" (4-sulphonamido-2-azo-7-acetylamino-1-hydroxynaphthalene-3:6-disulphonic acid) are split at the azo link and Fuller (1937) has isolated one of the split products, namely sulphanilamide, in crystalline form from the urine of patients treated with these drugs. In mice some 37·5% of " soluble prontosil " is excreted unchanged in 48 hours and 13·5% is broken down, giving rise to sulphanilamide. " Prontosil rubrum " is only slowly eliminated by mice and in 5 days only 5·8% of the dose is excreted unchanged with 2·3% broken down to sulphanilamide ; 90% is unaccounted for.



**4:4′Disulphonamidoazobenzene.** This compound is a chemical oxidation product of sulphanilamide and a possible biological reduction product of *p*-nitrobenzenesulphonamide (see p. 159). It is a compound of extremely low solubility in water and is likely to be poorly absorbed if administered orally. When injected into rats in aqueous suspension, it is excreted exceedingly slowly, for when a dose of 10 mg. is given to a rat only 14% can be accounted for in 6 days by its metabolites excreted in the urine. About 40% of that excreted is present as an amino compound (presumably sulphanilamide) and 75% of this latter is conjugated. It appears therefore that some 5% is converted to sulphanilamide in the rat (Flynn and Kohl, 1941).

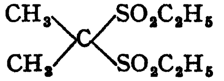


# *Chapter Ten*

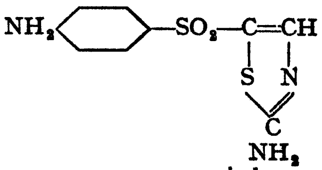
# The Metabolism of Sulphones, Sulphonic Acids and Sulphonamides

ORGANIC COMPOUNDS containing sulphur are extremely valuable in medicine and, of this class, the sulphones and sulphonamides are probably the most important. Aliphatic disulphones, such as sulphonal, are valuable as hypnotics and recently aromatic sulphones have been found possessing chemotherapeutic properties, for 4:4′-diaminodiphenyl sulphone appears to be active against streptococci and pneumococci in mice (cf. Findlay, 1939, for summary; Roblin, Williams and Anderson, 1941), and it also inhibits human tubercle *in vitro* (Smith, Emmart and Westfall, 1942). 2:4′-Diamino-5-thiazolylphenylsulphone (promizole) is a compound which contains sulphur additional to that in the sulphone group; it apparently possesses antitubercular activity for human tuberculosis in the guinea-pig (Feldman and Hinshaw cited by Bambas and Vandenbelt, 1944). Little is known as yet regarding the fate of such compounds in the animal body.

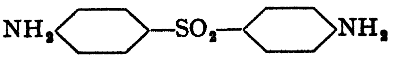
The aromatic sulphonamides are now widely used for the treatment of acute bacterial infections, and the discovery of their value in this respect has been one of the foremost advances of recent years.



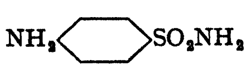
sulphonal



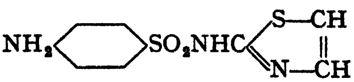
promizole



diaminodiphenylsulphone



sulphanilamide



sulphathiazole

**Aliphatic Disulphones.** There appears to be correlation between the hypnotic activity of the aliphatic disulphones and their metabolism in the body (Baumann and Kast, 1890). Those sulphones which are devoid of any activity are to a large extent excreted unchanged in the urine, whereas those which produce sleep undergo extensive biochemical transformation and little, if any, is excreted unchanged in the urine. The nature of the change which the active compounds undergo, however, is obscure. Baumann and Kast divided these sulphones into three groups:—

| | | |
|---|---|---|
| (*a*) | Methylene disulphones | $CH_2(SO_2R)_2$ |
| (*b*) | Methenyl disulphones | $CHR'(SO_2R)_2$ |
| (*c*) | Ketone disulphones | $CR'R''(SO_2R)_2$ |

where R, R′ and R″ are alkyl groups. Compounds of the first group are inactive and are mainly excreted unchanged by man and the dog. In the second group, some are active and others inactive and some 10% to 20% of the dose may be excreted unchanged. The third group includes such substances as sulphonal, trional and tetronal, and they are largely transformed *in vivo* to unknown metabolites,[1] only traces of the unchanged compounds being secreted in the urine. It is therefore clear that in some way the activity of the aliphatic sulphones is closely associated with their metabolism in the body. There is also a correlation between activity of the sulphone and the number of ethyl groups in the molecule (see Table 19).

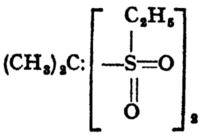
TABLE 19

*The Relationship between the Hypnotic Activity of the Alkyl Sulphones and their Degradation in the Body*

| Compound | Activity | Fate in the body |
|---|---|---|
| $(Et)_2SO_2$ | — | Mainly excreted unchanged |
| $CH_2(SO_2Me)_2$ | — | ,, ,, ,, |
| $CH_2(SO_2Et)_2$ | — | ,, ,, ,, |
| $CH_2SO_2Et$<br>$\vert$<br>$CH_2SO_2Et$ | — | ,, ,, ,, |
| $CHMe(SO_2Me)_2$ | — | About one-eighth excreted unchanged |
| $CHMe(SO_2Et)_2$ | + + | Only traces excreted unchanged |
| $CHEt(SO_2Me)_2$ | + | About one-sixth excreted unchanged |
| $CHEt(SO_2Et)_2$ | + + | Traces excreted unchanged |
| $CMe_2(SO_2Me)_2$ | — | None excreted |
| $CMeEt(SO_2Me)_2$ | + | Traces excreted |
| $CMe_2(SO_2Et)_2$ (Sulphonal) | + + | None excreted except after very large doses |
| $CEt_2(SO_2Me)_2$ | + + | ,, ,, ,, ,, |
| $CMeEt(SO_2Et)_2$ (Trional) | + + | ,, ,, ,, ,, |
| $CEt_2(SO_2Et)_2$ (Tetronal) | + + | ,, ,, ,, ,, |

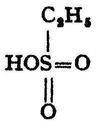
---

[1] Sulphonal is converted in the animal to an easily soluble organic sulphur compound which may be ethylsulphonic acid (Smith; see Kast, 1892). This compound (see p. 157), however, is not responsible for the toxic effects of sulphonal, for it is relatively non-toxic and does not cause haematoporphyrinuria as does sulphonal (Kast, 1892).



$$(CH_3)_2C{:}\left[-\overset{\overset{C_2H_5}{|}}{\underset{\underset{O}{\|}}{S}}{=}O\right]_2$$

**sulphonal**



$$HO\overset{\overset{C_2H_5}{|}}{\underset{\underset{O}{\|}}{S}}{=}O$$

**ethylsulphonic acid**

**Aromatic Sulphones.** Little is known as yet regarding the fate of the aromatic sulphones in the body. In the case of **promizole** (2:4′-diamino-5-thiazolylphenylsulphone) it is largely excreted by man as a water-soluble conjugate, in which substitution or conjugation has occurred in the thiazole ring. Patients receiving high doses of promizole excrete a coloured urine and apparently the compound responsible is a minor water-soluble metabolite of promizole, possessing a reddish-purple colour. Of the ingested promizole some 60–80% can be accounted for as a diazotisable amino compound (Bambas and Vandenbelt, 1944).

## THE METABOLISM OF THE SULPHONIC ACIDS AND SULPHONAMIDES

One of the most striking advances made in the field of chemotherapy was the discovery by Domagk in 1935 of the remarkable curative properties of the red azo dye, prontosil, against streptococcal infections in mice. This discovery led to the synthesis and testing of a whole host of compounds containing the sulphonamide group, for activity against acute bacterial infections. The most active compounds were found to be derivatives of *p*-aminobenzenesulphonamide or sulphanilamide, and today sulphanilamide, M and B 693, sulphathiazole are almost household words. Despite the great success of these drugs in a large number of acute bacterial infections, and the tremendous advances on the clinical side, their fate in the animal body has not been definitely cleared up. There is no doubt that in a few cases the major proportion of the drug can be accounted for by acetylation, but even so the lesser proportion not accounted for may be of importance in partly explaining some of the toxic effects of these drugs.

**The Aromatic Sulphonic Acids.** From therapeutic activity to therapeutic inertness is often a very short step as far as chemical structure is concerned, for a slight alteration in a single chemical group often renders an active compound inactive. It is not surprising, therefore, that whilst a number of sulphonamides are therapeutically active, their corresponding sulphonic acids are as a rule either very feebly active [1] or completely inactive. That their inactivity is related to any differences in their metabolism as compared with the sulphonamides is unlikely in view of the present theories of sulphonamide activity. The fate of some of the sulphonic acids in the animal body has received a little attention. It appears that most of these compounds are excreted unchanged by the animal organism. This probably applies to the aliphatic as well as aromatic sulphonic acids for Flaschenträger, Bernhard, Löwenberg and Schläpfer (1934) have shown that ***n*-octanesulphonic acid** is excreted unchanged to the extent of 83% by the dog. **Ethylsulphonic acid** is mainly excreted unchanged, but **hydroxyethylsulphonic acid** is completely oxidised by the rabbit (Salkowski, 1876).

---

[1] Sulphanilic and metanilic acids are slightly active (Buttle, Gray and Stephenson, 1936).

**Benzenesulphonic acid** injected into dogs can be recovered unchanged in the urine (Salkowski, 1871).

*p*-**Hydroxybenzenesulphonic acid** (phenol-*p*-sulphonic acid) is used as an antiseptic under the name "aseptol". When fed to an animal it is mainly excreted unchanged (Baumann and Herter, 1877). It does not form an ethereal sulphate in the rabbit despite the fact that it possesses a phenolic hydroxyl (Sammons, Shelswell and Williams, 1941).

*p*-**Aminobenzenesulphonic acid** or **sulphanilic acid** is excreted unchanged according to Hildebrandt (1906), but Zehender (1943) finds that it is acetylated in the guinea-pig, 75% of the excreted sulphanilic acid being in the acetylated form. The aminophenolsulphonic acids, **1-amino-2-hydroxy-, 1-amino-3-hydroxy-** and **1-hydroxy-2-aminobenzene-4-sulphonic acids** (Sammons *et al.*, 1941), are probably excreted unchanged.

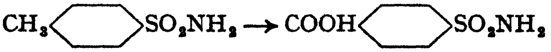
## THE AROMATIC SULPHONAMIDES

Chemically, the aromatic sulphonamide group is fairly stable and is not easily split off. There is no evidence that it can be split off *in vivo*, for the nuclear carbon to sulphur link is biologically stable.

The sulphonamide group is also resistant to hydrolysis to a sulphonic acid for fairly strong acids are usually required for the process. There are, however, some sulphonamides which are fairly easily hydrolysed, for Fosbinder and Walter (1939) have reported that if 2-$N^4$-acetylsulphanilamido-6-aminopyridine is refluxed with 10% HCl it yields sulphanilic acid, whereas 36% HCl is required to hydrolyse sulphapyridine to sulphanilic acid (Crossley, Northey and Hultquist, 1940). There is no evidence to show that the sulphonamide group can be converted into a sulphonic acid *in vivo*. The aromatic $—SO_2NH_2$ group appears to resist transformation *in vivo* and is usually excreted as such.

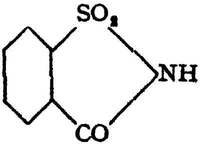
**Benzenesulphonamide.** Little is known regarding the fate of the simplest compound of this series, namely, benzenesulphonamide, but it appears to be a relatively toxic body.

*p*-**Toluenesulphonamide** is converted and excreted by the dog mainly as *p*-sulphonamidobenzoic acid (Flaschenträger *et al.*, 1934). The oxidation of the methyl group is probably the only change it undergoes, for Sammons *et al.* (1941) have shown that in rabbits it does not increase the output of ethereal sulphate and is therefore unlikely to be oxidised to a phenol.



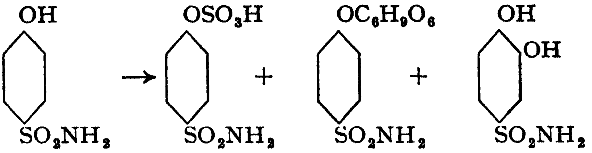
It is slightly active against streptococcal infections on mice (Buttle *et al.*, 1936).

**Saccharin**, the well-known sweetening agent, is the anhydride of *o*-sulphonamidobenzoic acid,



Its wide use, particularly in war-time, as a sugar substitute should elicit interest in what happens to it in the body. Its fate in the body is, however, quite uninteresting. It is said to be excreted entirely unchanged in man and its excretion is fairly rapid, small doses being completely eliminated in 24 hours and large doses within 48 hours. In the rabbit, after oral or parenteral administration, 70–80% appears in the urine unchanged (see Heffter, 1934). There is no record that any of it undergoes change.

**$p$-Hydroxybenzenesulphonamide** behaves in the rabbit as a typical phenol. The sulphonamide group is excreted unchanged whilst the hydroxyl group conjugates with sulphuric and glucuronic acids. In addition a small proportion is oxidised to catechol-4-sulphonamide, which is also excreted conjugated (Sammons *et al.*, 1941; Williams, 1941).



Both the glucuronide and the catecholsulphonamide have been isolated from the urine of rabbits, the former as barium $p$-sulphonamidophenyl-glucuronide ($[\alpha]_D$ −50°) and the latter as the tetramethyl derivative, veratrol-4-sulphondimethylamide (m.p. 115°).

**$p$-Nitrobenzenesulphonamide** has a high antistreptococcal activity *in vivo* and organisms of the *Neisseria*, *Bacterium* and *Clostridium* groups are also markedly sensitive to it *in vitro*. When fed to rats it is largely reduced to sulphanilamide and excreted mainly as acetylsulphanilamide. Some 82–83% is excreted in three days and most of this appears on the first day. Of the amount excreted about 10% appears as a free amine, about 80% as a conjugated amine and 10% as material not reduced to the amine (Flynn and Kohl, 1941).

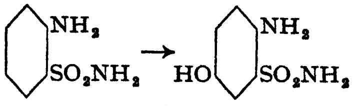
## AMINOBENZENESULPHONAMIDE AND ITS DERIVATIVES

The sulphonamide derivatives dealt with so far are mainly therapeutically inactive. The presence of the amino group attached to the benzene ring *para* to the sulphonamide group produces therapeutic activity, yet if the amino group is in the *meta-* or *ortho-* position inactive compounds are obtained.

Orthanilamide and metanilamide are chemotherapeutically inactive although they can, like sulphanilamide itself, inhibit the respiration of resting cells of *E. coli*, *Staph. aureus* and *Staph. pyogenes* (Wyss, Strandskov and Schmelkes, 1942).

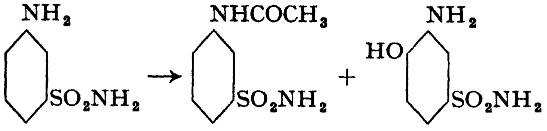
**Orthanilamide** is acetylated only to a small extent in the rabbit. More than a half is oxidised to a phenol, probably 2-amino-5-hydroxybenzenesulphonamide, which is excreted conjugated whereas about 40%

is excreted unchanged (Williams, 1945). Some 40% of orthanilamide fed to rabbits is excreted as the ethereal sulphate of hydroxyorthanilamide



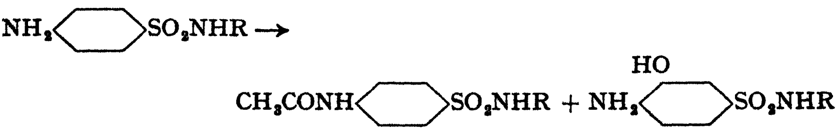
hydroxyorthanilamide

**Metanilamide** is acetylated to a considerable extent in the rabbit and about 50% makes its appearance as acetylmetanilamide. Some 20% is excreted as an ethereal sulphate, and a glucuronide is also excreted. The excretion of ethereal sulphate and conjugated glucuronic acid indicates that metanilamide is oxidised to a phenol which is 3-amino-4-hydroxybenzenesulphonamide (Dobson and Williams, 1946).



## SULPHANILAMIDE AND ITS DERIVATIVES

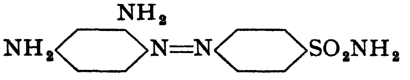
In 1937 Mayer suggested that the activity of **sulphanilamide** was due to one of its biological oxidation products and postulated the slow formation of *p*-hydroxylaminobenzenesulphonamide as the basis for sulphanilamide activity. This theory has now been superseded by that of Woods and Fildes which postulates that sulphanilamide acts by competing with *p*-aminobenzoic acid, an essential metabolite for certain bacteria. The suggestion of Mayer, however, stimulated an interest in the fate of the sulphonamides in the animal body. In general sulphanilamide and its derivatives such as **sulphapyridine, sulphathiazole,** etc., that is, compounds in which the nuclear amino group is unsubstituted, are partly excreted unchanged, partly acetylated (forming N$^4$-acetyl compounds) and partly oxidised to phenolic compounds which may be excreted combined with sulphuric and glucuronic acids, thus :—



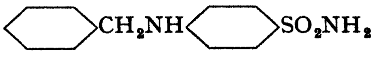
N$^4$-acetyl derivative

R = H or another group : the position of the OH group is arbitrary

The group R may also be oxidised, as in the cases when R is pyridine and thiazole. The extent to which the drug is excreted unchanged, acetylated and oxidised, however, depends on the nature of the substituent R. Drugs like **prontosil** and **benzylsulphanilamide**, where the nuclear $NH_2$ has been substituted or modified, are first converted *in vivo* to sulphanilamide, which then follows its normal metabolism (Tréfouel, Tréfouel, Nitti and Bovet, 1936; Fuller, 1937; James and Fuller, 1940).

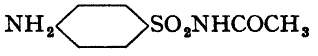


prontosil



benzylsulphanilamide (M & B 126)

N$^1$-Acylsulphanilamides are partly deacylated by the dog, although **N$^1$-acetylsulphanilamide** after oral administration is excreted unchanged by this animal (Robinson and Crossley, 1943).



N$^1$-acetylsulphanilamide

**Acetylation.** Acetylation of sulphanilamide occurs in all animals (fish, frog,[1] toad, chicken, mouse, rat, guinea-pig, rabbit, cat, pig, cow, horse, monkey, man) except the dog, the turtle and certain species of frog[1] (Marshall, 1939; Marshall, Emerson and Cutting, 1937; Failey, Anderson, Henderson and Chen, 1943). In the dog it is largely excreted unchanged, for Marshall, Emerson, Cutting and Babbit (1937) claim that a dog may excrete 78–95% unchanged. The acetylation occurs to a greater extent with sulphanilamide than with sulphapyridine, sulphathiazole or sulphadiazine. The extent of acetylation (which is a very variable process) of the aromatic amino group in various sulphanilamide derivatives is given in Table 20.

Acetylated sulphanilamide derivatives are less soluble than the free drugs, and unless a high fluid intake is maintained during treatment, the acetyl compounds tend to crystallise *in vivo*, often injuring the kidney and producing crystalluria. The solubilities and melting-points of the better-known sulphonamides and their N$^4$-acetyl[2] derivatives are given in Table 21 (solubilities mainly after Marshall *et al.*, 1940, and Hawking and Green, 1945).

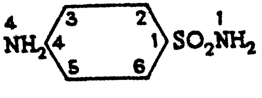
N$^4$-Acetylsulphanilamide is also more toxic than sulphanilamide, for its median lethal dose (L.D. 50) for mice is 2–3 g./kg. (Marshall, Cutting and Emerson, 1938), whilst that for sulphanilamide is 3–4 g./kg. (3·8 g./kg. Marshall *et al.*, 1938; 4 g./kg., Dyke, 1937). It is obvious that in this case the acetylation of sulphanilamide *in vivo* cannot be considered as a true detoxication process.

**Oxidation.** The extent to which sulphanilamide derivatives are oxidised *in vivo* is, like acetylation, dependent upon the nature of the substituent attached to the sulphonamide amino group.

---

[1] The spadefoot toad (*Scaphiopus holbrookii holbrookii*), the nebulous toad (*Bufo valliceps*) and the tree frog (*Hyla cinerea cinerea*) conjugate most sulphonamides. The leopard frog (*Rana pipiens*), the "Jumbo" bullfrog (*Rana catesbeiana*) and the turtle (*Pseudemys elegans*) do not conjugate sulphonamides (Failey *et al.*, 1943).

[2]



### TABLE 20

*The Acetylation of Sulphanilamide and Related Compounds* in vivo

| Compound | Species | Dose | Time of excretion | Total excreted % of dose | Approx. amount acetylated % of total excreted | Reference [6] |
|---|---|---|---|---|---|---|
| **Sulphanilamide** | man | 30·9 g. in 8 days | 10 days | 86–87 | 60 | Marshall *et al.* (1937) |
| ,, | rabbit | 0·1 g./kg. | 2 ,, | 70–80 | 90 | ,, ,, ,, |
| ,, | dog | various | 2 ,, | 78–95 | 0 | ,, ,, ,, |
| **Sulphapyridine** [1] | man | 16·5 g. in 7 days | 10 ,, | 82·5 | 50 | Stokinger (1939) |
| ,, | dog | 2 g. | 2 ,, | *ca.* 100 | 0 | ,, ,, |
| **Sulphathiazole** [2] | man | 2 g. | 2 ,, | 80–85 | 25 | Zehender (1943) |
| ,, | guinea-pig | 0·19 g./kg. | 10 hours | 25 | 35 | ,, ,, |
| **Acetylsulphathiazole** | man | 2 g. | 2 days | 9 | 82 | ,, ,, |
| ,, | guinea-pig | 0·19 g./kg | 10 hours | 5 | 100 | ,, ,, |
| **Sulphaguanidine** [3] | man | 0·1 g./kg. | 24 ,, | 10–40 | 50 | Marshall *et al.* (1940) |
| ,, | dog | 0·1 g./kg. | 24 ,, | 60–70 | 0 | ,, ,, ,, |
| **Sulphadiazine** [4] | man | 5 g. | 3 days | 75 | 30 | Peterson *et al.* (1941) |
| **Sulphamethazine** [5] | man | — | — | *ca.* 100 | *ca.* 90 | Sykes (1944) |
| **Acetylsulphamethazine** | man | — | — | *ca.* 100 | *ca.* 99 | ,, ,, |
| **$p$-Aminobenzoic acid** | man | 1 g. | 24 hours | 75 | 68 | Zehender (1943) |
| ,, ,, | guinea-pig | 0·1 g./kg. | 10 ,, | 58 | 92 | ,, ,, |
| **$p$-Acetaminobenzoic acid** | man | 1 g. | 24 ,, | 63 | 100 | ,, ,, |
| ,, ,, | guinea-pig | 0·1 g./kg. | 10 ,, | 27 | 100 | ,, ,, |
| **Aniline hydrochloride** | guinea-pig | 0·1 g./kg. | 8 ,, | 41 | 20 | ,, ,, |
| **Sulphanilic acid** | guinea-pig | 0·14 g./kg. | 10 ,, | 56 | 75 | ,, ,, |

[1] 2-Sulphanilamidopyridine.

[2] 2-Sulphanilamidothiazole.

[3] Sulphanilylguanidine.

[4] 2-Sulphanilamidopyrimidine.

[5] 2-Sulphanilamido-3:6-dimethylpyrimidine.

[6] The figures quoted in the above table have been taken at random from the literature.

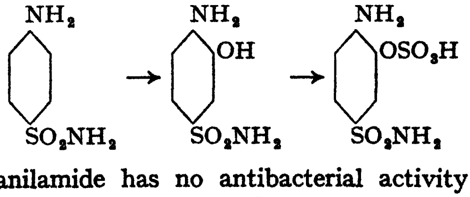
TABLE 21

*Solubilities and Melting-points of some Sulphonamide Drugs at 37° and pH ca. 7*

| Compound | M.p. | Solubility in mg./100 c.c. | |
|---|---|---|---|
| | | In water | In urine |
| Sulphanilamide | 165° | 1,480 | — |
| $N^4$-Acetylsulphanilamide | 218° | 534 | — |
| Sulphaguanidine [1] | 189–190° | 220 | 218 |
| $N^4$-Acetylsulphaguanidine | 264–267° | 40 | 48 |
| Sulphapyridine | 190–191° | 54 | 57 |
| $N^4$-Acetylsulphapyridine | 230–231° | 16 | 19 |
| Sulphathiazole [2] | 172–175° (202°) | 96 | — |
| $N^4$-Acetylsulphathiazole | 258° | 6 | — |
| Sulphadiazine | 251–252° | 12 | — |
| $N^4$-Acetylsulphadiazine | 254–255° | 15 | — |
| Sulphamerazine | 235–236° | 40 | — |
| $N^4$-Acetylsulphamerazine | 244° | 24·7 | 27 |
| Sulphamethazine [3] | 175–176° | 235 | — |
| $N^4$-Acetylsulphamethazine | 240–241° | 130 | — |

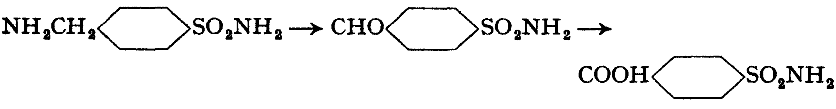
[1] Monohydrate, m.p. 142·5–143·5°.
[2] Sulphathiazole is dimorphic (Leech, 1941).
[3] Hemihydrate, m.p. 197–199°.

Shelswell and Williams (1940) have shown that in rabbits **sulphanilamide** causes an increased output of ethereal sulphates corresponding to about 6–12% of the dose. This indicates that sulphanilamide is oxidised *in vivo* to a phenol which has now been isolated and identified as 3-hydroxysulphanilamide (Williams, 1946). The same phenol occurs in a conjugated form in the urine of patients treated with sulphanilamide (Williams and Ansell, 1945). It is probable that no glucuronic acid conjugate is formed from sulphanilamide in rabbits and rats (Williams, 1946; Scudi and Robinson, 1941). The oxidation of sulphanilamide, which is a minor reaction in the metabolism of the drug, is probably as follows:—

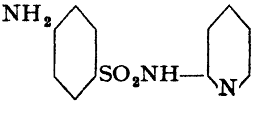


3-Hydroxysulphanilamide has no antibacterial activity *in vivo* when tested in mice infected with haemolytic streptococci, although it shows some activity *in vitro*. These results indicate that the activity of sulphanilamide cannot be ascribed to oxidation products as postulated by Mayer (1937) and others. The identification of 3-hydroxysulphanilamide as the oxidation product of sulphanilamide also disposes of those theories which held that *p*-hydroxylaminobenzenesulphonamide was the oxidation product (cf. James, 1940; Rosenthal, 1939). ***p*-Hydroxylaminobenzenesulphonamide** is rapidly reduced *in vivo* to sulphanilamide which is excreted as such in the dog (Bratton *et al.*, 1939) and partly as $N^4$-acetylsulphanilamide in the rabbit (Williams, 1946).

**Homosulphanilamide** (*p*-aminomethylbenzenesulphonamide, " Marfanil ") , unlike sulphanilamide, is not inhibited by *p*-aminobenzoic acid, pus and necrotic tissue. It is therefore very useful in the treatment of surface wounds where the activity of sulphanilamide could be inhibited by pus, etc. In the body, however, Marfanil is inactive. This systemic inactivity is probably due to its rapid transformation into inactive metabolites. Hartles and Williams (1946) have shown that in the rabbit a 2 g. dose of Marfanil is transformed in 24 hours to the extent of 70% to *p*-sulphonamidobenzoic acid. Marfanil is a substrate for amine oxidase (Blaschko and Duthie, 1945) which probably transforms it to *p*-aldehydobenzenesulphonamide. This latter is then oxidised to *p*-sulphonamidobenzoic acid thus :—



**Sulphapyridine** is oxidised in the dog to a hydroxysulphapyridine (m.p. 180–181°), which is excreted as a glucuronide. The silver salt of this glucuronide, $C_{11}H_9O_2N_3SAg.OC_6H_8O_6Ag,3H_2O$, has been isolated by Scudi (1940). Hydroxysulphapyridine glucuronide has also been isolated from dog urine by Weber, Lalich and Major (1943), who have shown that the hydroxyl group formed by oxidation *in vivo* is present in the heterocyclic ring and not in the benzenoid ring. The location of this OH group in the pyridine ring, however, has not been ascertained.



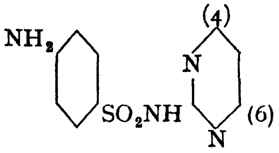
sulphapyridine

In the rat nearly 40% of the sulphapyridine excreted in the urine may be present as a water-soluble glucuronide (Scudi and Robinson, 1941).

The pyridine ring of sulphapyridine is not methylated in man as might be expected (James, 1940).

It appears probable that **sulphathiazole** undergoes appreciable oxidation in rabbits, for in these animals it gives rise to considerable amounts of conjugated glucuronic acid (Thorpe and Williams, 1940). The nature of the oxidation product is, however, obscure.

**Sulphadiazine** only undergoes oxidation in the rabbit to a very slight extent. Its 4-methyl and 4:6-dimethyl derivatives, **sulphamerazine** and **sulphamezathine,** are both more highly acetylated and oxidised in man and in the rabbit than sulphadiazine itself (Smith and Williams, unpublished). Sulphamerazine and sulphamezathine both increase the output of glucuronic acid in man, whereas sulphadiazine does not (Gilligan, 1945).



sulphadiazine

*Chapter Eleven*

# The Metabolism of Terpenes and Camphors

THE terpenes as a whole are a fascinating class of compounds from a purely chemical point of view, and no less can be said about them from the biochemical aspect. The changes undergone in the animal body by terpenes and their derivatives are often unexpected and therefore interesting. Some terpene derivatives are of great antiquity: thus common or Japan camphor is probably one of the oldest known organic compounds. Since many terpenes are natural products found in essential oils and in a variety of herbs and plants used in medicine or for culinary purposes, it is reasonable to suppose that small amounts of terpenes are continually being absorbed and metabolised by man during life. These small amounts no doubt contribute to a slight extent to the normal output of glucuronic acid in the urine, although there is no record in the literature of terpene derivatives and their conjugated glucuronic acids occurring in normal urine.[1] *l*-Borneol has been found in the contents of the perfume gland of the beaver, its origin being probably the plant material eaten (Walbaum and Rosenthal, 1927).

## THE FATE OF OPEN-CHAIN OLEFINIC TERPENES

Open-chain terpenes can usually be represented by the formulae $C_{10}H_{16}$, $C_{10}H_{16}O$ or $C_{10}H_{18}O$. Strictly speaking, they belong to the aliphatic series, but they are closely related chemically to the terpenes proper and the camphors, with which they are found in natural essential oils. The scents of the oils of geraniums, lemons, lavender, bergamot, coriander and linaloes and attar of roses are due to the presence of open-chain terpenes.

It is well known that aliphatic straight-chain compounds undergo oxidation in the body according to the theory of $\beta$-oxidation and $\omega$-oxidation. In the rabbit the open-chain terpenes undergo $\omega$-oxidation. Other forms of oxidation such as $\beta$- and $\delta$-oxidation probably do not occur. Reduction of the $\alpha$:$\beta$-double bond may also take place.

Kuhn, Köhler and Köhler (1936; cf. Kuhn and Livada, 1933) studied the fate in the rabbit of a series of open-chain terpene derivatives which comprised hydrocarbon, alcohol, aldehyde and acid, namely, dihydromyrcene, geraniol, citral (geranial) and geranic acid.

**Dihydromyrcene** in the rabbit undergoes a double $\omega$-oxidation with the production of 2:6-dimethyl-$\Delta^{1:5}$ hexadiene-1:6-dicarboxylic acid. The acid was first described as early as 1901 by Hildebrandt (1901*a*), who obtained it from the urine of dogs and rabbits after the administration of citral, and it has since been called " Hildebrandt acid ".

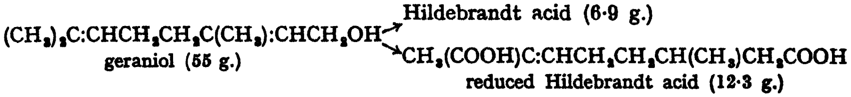
---

[1] It would be interesting to know the glucuronic acid and terpene content of the urine of the Australian teddy bear (Koala bear) which lives on eucalyptus leaves.

$$\underset{\text{dihydromyrcene}}{(CH_3)_2C{:}CHCH_2CH_2C(CH_3){:}CHCH_3} \longrightarrow \underset{\text{Hildebrandt acid}}{CH_3(COOH)C{:}CHCH_2CH_2C(CH_3){:}CHCOOH}$$

Kuhn *et al.* (1936) isolated 1·5 g. of this acid after feeding 25 g. of dihydromyrcene, and no other metabolites were detected. Geraniol, citral and geranic acid are also metabolised to this acid, but in these cases other metabolites are also formed.

**Geraniol** (inactive) not only undergoes a double $\omega$-oxidation but also an asymmetric reduction of the 2:3 double bond, the 6:7 double bond remaining unaltered. The products of geraniol metabolism are therefore " Hildebrandt acid " and 2:6-dimethyl-$\Delta^5$-hexene-1:6-dicarboxylic acid. The latter acid is optically active ($[\alpha]_D + 15°$ for Na salt).

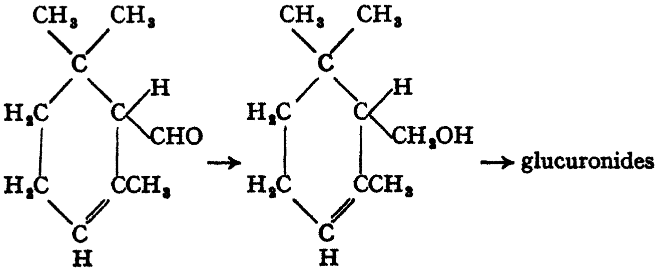


$$\underset{\text{geraniol (55 g.)}}{(CH_3)_2C{:}CHCH_2CH_2C(CH_3){:}CHCH_2OH} \begin{cases} \nearrow \text{Hildebrandt acid (6·9 g.)} \\ \searrow \underset{\text{reduced Hildebrandt acid (12·3 g.)}}{CH_3(COOH)C{:}CHCH_2CH_2CH(CH_3)CH_2COOH} \end{cases}$$

**Citral,** $(CH_3)_2C{:}CHCH_2CH_2C(CH_3){:}CHCHO$, is metabolised to the same acids as is geraniol. Citral is of special interest since it has the property of inhibiting tumours. Boyland (1940) has shown that one of its metabolites, " Hildebrandt acid ", is much less active in this respect than citral (see p. 27).

**Geranic acid,** $(CH_3)_2C{:}CHCH_2CH_2C(CH_3){:}CHCOOH$ (20 g.), also undergoes $\omega$-oxidation to " Hildebrandt acid " (2·3 g.) and reduced " Hildebrandt acid " (1·5 g.), but in this case relatively more of the former is produced than in the case of geraniol.

The cyclised compounds, *cyclo***citral** and *cyclo***geraniol** when fed to rabbits do not form " Hildebrandt acid ", instead they are excreted as glucuronides. The glucuronide excreted in both cases is probably that of *cyclo*geraniol (Hildebrandt, 1904).



*cyclo*citral *cyclo*geraniol

Now the question might be asked why does β-oxidation not occur during the metabolism of these compounds, since it is known that unsaturated acids of the type R.CH:CHCOOH and β-methyl acids, $RCH(CH_3)CH_2COOH$ undergo β-oxidation. The answer to this question was supplied by Kuhn *et al.* (1936), who showed that a combination of the α:β double bond with a β-methyl group (or an α-methyl) resists biological oxidation. Thus in rabbits β-methyl-β-*n*-hexylacrylic acid undergoes ω-oxidation in rabbits :—

$$CH_3(CH_2)_5C(CH_3){:}CHCOOH \rightarrow COOH(CH_2)_5C(CH_3){:}CHCOOH$$

whereas β-methyl-β-*n*-nonylacrylic acid undergoes ω-oxidation and three β-oxidations producing 2-methyl-$\Delta^1$-butene-1:4-dicarboxylic acid.

$$CH_3(CH_2)_8C(CH_3){:}CHCOOH \rightarrow COOH(CH_2)_2C(CH_3){:}CHCOOH$$

It will be noted that oxidation takes place at the opposite end of the molecule to the methyl acrylic group.

In the case of *dl*-β-methylstearic acid, $CH_3(CH_2)_{14}CH(CH_3)CH_2COOH$, in which the α:β double bond is non-existent, the acid is completely oxidised *in vivo.*

In this work the interest of Kuhn and his co-workers lay in the mechanism of biological oxidation of the —$CH_3$ group to the —COOH group. Their views are that the biological oxidation of $CH_3$ to COOH cannot be performed in one step but in three steps, probably *via* the primary alcohol, —$CH_2OH$, and the aldehyde, —CHO. In the aliphatic terpenes the stage —$CH_3 \rightarrow$ —$CH_2OH$ cannot be detected, but as will be seen later, in the cyclic terpenes this stage can be detected for the alcohol forms conjugates with glucuronic acid which can be isolated. Kuhn and his co-workers point out that the stage —$CH_2OH \rightarrow$ —CHO is never detected *in vivo* for the —CHO group is easily oxidised or, in some cases, reduced.

**Citronellal.** This terpene aldehyde is the chief constituent of citronella oil and the oil of *Eucalyptus maculata*, and is frequently found with citral in lemon and rose oils. It will be noted that the acid (reduced " Hildebrandt acid ") obtained by reduction of citral in the rabbit is closely related to citronellal.

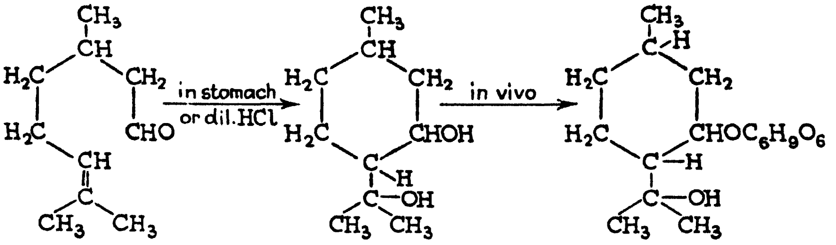
$$(CH_3)_2C{:}CHCH_2CH_2CH(CH_3)CH_2CHO$$

citronellal

$$CH_3(COOH)C{:}CHCH_2CH_2CH(CH_3)CH_2COOH$$

(acid from biological reduction of citral, geraniol and geranic acid)

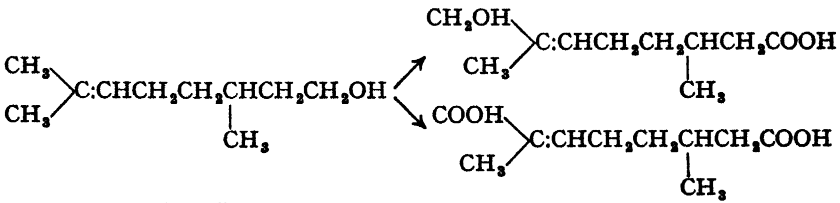
It could be expected that on feeding citronellal to rabbits this acid would be formed in appreciable amounts since the only processes involved are oxidation of the —CHO group and of one of the ω—$CH_3$ groups. Kuhn and Löw (1938) found that this did not happen and a quite unexpected change occurred. On feeding 50 g. citronellal to rabbits there was isolated 13 g. of a crystalline glucuronide which proved to be p-menthan-3:8-diol-*d*-glucuronide. Citronellal appears to have been cyclised in the rabbit and the glucuronide obtained was identical with that obtained on feeding **p-menthan-2:8-diol** (menthoglycol).



Kuhn and Löw, however, produced evidence to show that the cyclisation of citronellal was not, strictly speaking, a biological reaction, but a purely chemical one which took place in the stomach under the influence of the gastric hydrochloric acid. The conjugation of the menthoglycol with glucuronic acid was of course a purely biological reaction. Kühn and Löw found that on shaking 20 g. of citronellal with 200 c.c. of 0·5% HCl (stomach concentration) for 48 hours at 37°, 12 g. of menthoglycol were formed.

In menthoglycol glucuronide, the uronic acid residue is attached to the secondary alcohol group and not to the tertiary OH in position 8, which is free in the glucuronide. On acetylation with pyridine and acetic anhydride the methyl ester of the glucuronide takes up three acetyl groups, the tertiary hydroxyl of menthoglycol itself is not acetylated under similar conditions.

**Citronellol** (rhodinol), the primary alcohol corresponding to citronellal, does not undergo cyclisation (Fischer and Bielig, 1940). The main products of its metabolism in the rabbit are 2:6-dimethyl-$\Delta^5$-hexene-1:6-dicarboxylic acid and 2:6-dimethyl-7-hydroxy-$\Delta^5$-heptene-1-carboxylic acid, both of which are excreted in optically active forms. The latter acid is obviously the precursor of the former.

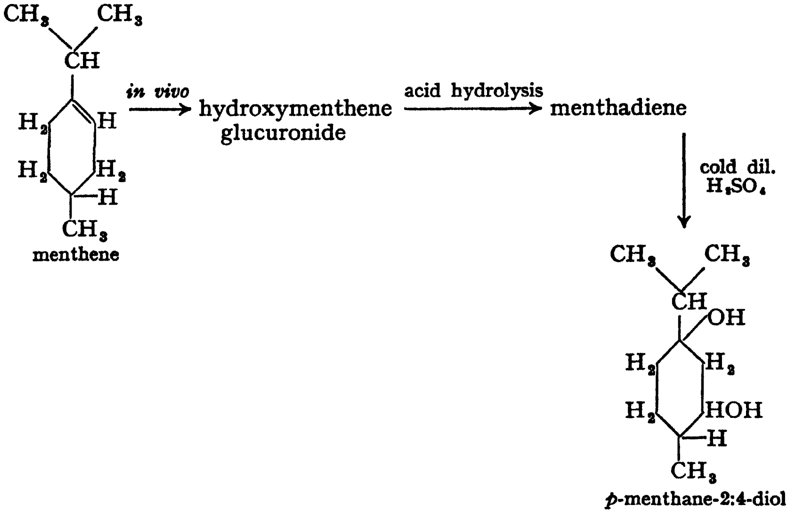


citronellol

## MONOCYCLIC TERPENES

### Hydrocarbons

**p-Menthene.** The only hydrocarbons of the monocyclic terpenes that have been studied appear to be *p*-menthene (Hämäläinen, 1912) and α-phellandrene (Harvey, 1942). In rabbits *p*-menthene forms a conjugated glucuronic acid of obscure constitution. Nevertheless, the formation of this glucuronide indicates that menthene is oxidised *in vivo.* On hydrolysis with acid the glucuronide is converted into a menthadiene which can be hydrated to *p*-menthane-2:4-diol by cold dilute sulphuric acid.



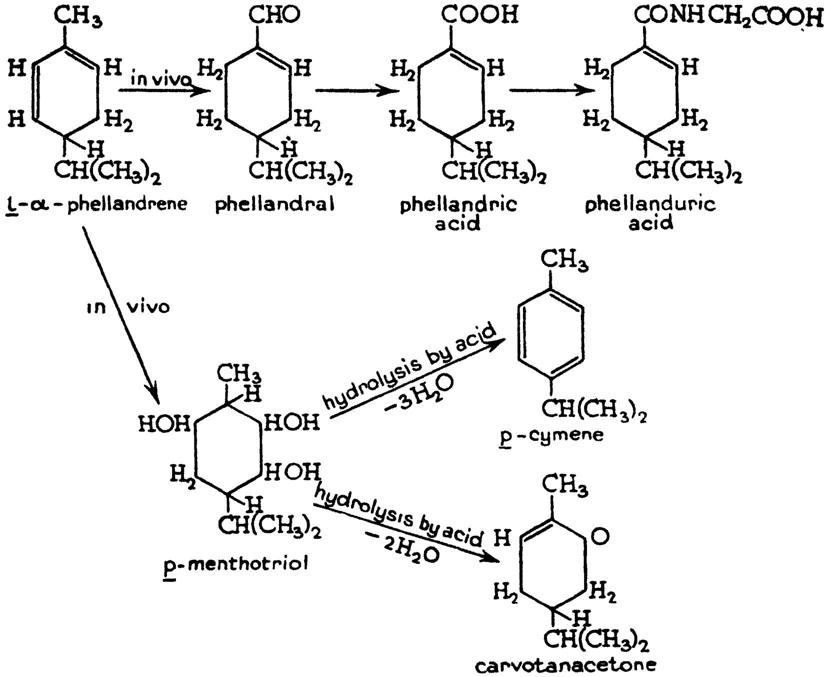
α-**Phellandrene** ($\Delta^{1:5}$-*p*-menthadiene). It appears probable that in the sheep the methyl group of α-phellandrene is oxidised to carboxyl, and one or both of the double bonds is reduced. Harvey (1942) fed 10 c.c. of α-phellandrene to a sheep, then hydrolysed and steam-distilled the resulting urine. The distillate contained phellandric acid, carvotanacetone ($\Delta^2$-menthene-6-one), *p*-cymene and possibly phellandral. It is probable that *p*-cymene and carvotanacetone were artefacts.

Phellandric acid is partly excreted as a glycine conjugate, phellanduric acid, which appears to be the first conjugate to be described in which glycine is combined with a carboxyl group attached to a tertiary carbon atom (Wright, 1945). Wright has also provided a feasible explanation of the occurrence of *p*-cymene and carvotanacetone amongst the hydrolysis products of phellandrene urine from sheep. He suggests that α-phellandrene is reduced *in vivo* to trihydroxy-*p*-menthane which is excreted as a glucuronide. On heating with acid, the loss of three molecules of water from the triol would yield *p*-cymene and the loss of two would yield carvotanacetone.

The work of Harvey and of Wright indicate that the metabolism of *l*-α-phellandrene in the sheep takes the course as shown on p. 171.

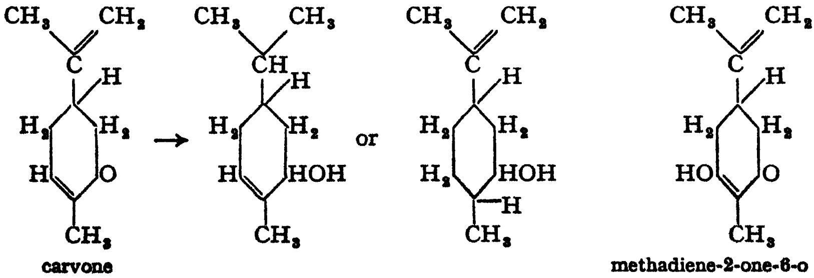
## Ketones

The biological behaviour of the keto group in monocyclic terpenes is similar to that in aromatic ketones (see p. 88) and unlike that of



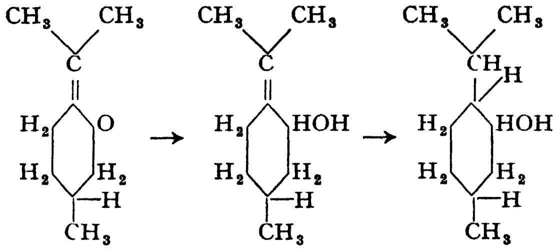
the ketones of the bicyclic terpenes (see p. 179). Reduction of the keto group takes place with the production of a secondary alcohol which is excreted conjugated with glucuronic acid.

**Carvone.** Carvone ($\Delta^{6:8(9)}$-menthadiene-2-one) is the chief constituent of the oil of caraway seeds. Hildebrandt (1902) showed that it formed a glucuronide in the rabbit. The more recent work of Fischer and Bielig (1940) has shown that it is converted in the rabbit to a dextrorotatory carbinol in which one ethylenic link is saturated and the keto group is reduced. This alcohol may be $\Delta^{6}$- or $\Delta^{8(9)}$-menthene-2-ol.



A keto-alcohol closely related to carvone, namely **$\Delta^{6:8(9)}$-menthadiene-2-one-6-ol** is excreted by rabbits combined with glucuronic acid but otherwise unchanged (Hildebrandt, 1902).

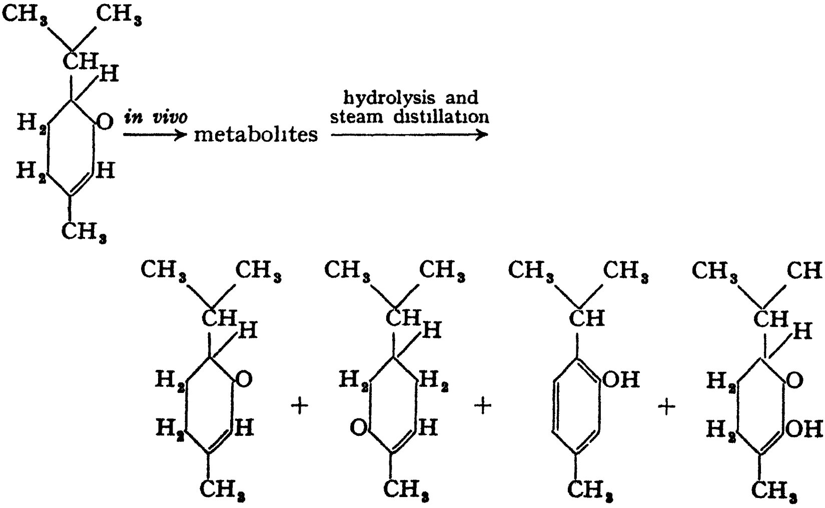
**$d$-Pulegone** ($\Delta^{4(8)}$-$p$-menthene-3-one) on oral administration to rabbits is converted to $d$-menthol and $l$-pulegol, both being excreted as glucuronides (Teppati, 1937 ; cf. Hildebrandt, 1902).



pulegone pulegol menthol

The formation of pulegol by reduction of the keto groups is obviously the first stage in the reduction ; pulegol is then reduced at its double-bond forming menthol. Both reductions are probably asymmetric, since reduction of pulegone could give two pulegols and reduction of each pulegol could be expected to give to two menthanols.

**Piperitone** ($\Delta^1$-$p$-menthene-3-one). When piperitone is fed to sheep and the resulting urine hydrolysed and steam-distilled, the distillate contains piperitone, carvotanacetone, thymol and possibly diosphenol (buchu-camphor) (Harvey, 1942)

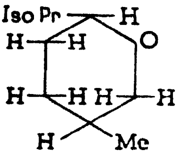


carvotanacetone thymol diosphenol

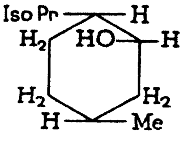
All the products contain the 1:2-double bond characteristic of piperitone, but it is difficult from their nature to draw any conclusions regarding the metabolism of piperitone.

**$l$-Menthone** ($p$-menthane-3-one). Both Neubauer (1901) and Bonnani (1902) showed that $l$-menthone gave rise in rabbits to conjugated glucuronides. Hämäläinen (1912) claimed that menthone was probably reduced to ketomenthone in the rabbit since the glucuronide produced was converted by warm dilute $H_2SO_4$ to a substance which was probably $\Delta^{4(8)}$-*p*-menthen-3-one (pulegone).

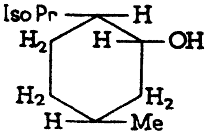
More definite results were obtained by Williams (1940), who showed that *l*-menthone underwent reduction in the rabbit with the production of *d*-*neo*menthol. The presence of *l*-menthol, the other possible reduction product, was not detected and therefore it was assumed that the biological reduction could be asymmetric.



menthone



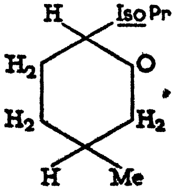
*neo*menthol



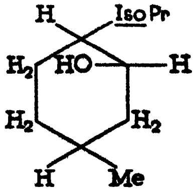
menthol

Williams points out that there are two possible explanations of the appearance of *d*-*neo*menthol without *l*-menthol during the biological reduction of *l*-menthone :—(1) During the biological reduction of *l*-menthone, the production of the new optically active carbon atom is a completely asymmetric process, *d*-*neo*menthol only being formed. (2) The biological reduction may produce both *d*-*neo*menthol and *l*-menthol in equal or unequal quantities, but the rate of further oxidation of *l*-menthol or its glucuronide is greater than that of *l*-neomenthol and its glucuronide and is such that no *l*-menthol survives *in vivo*. The second of these explanations receives some support from the fact that when pure *d*-*neo*menthol is fed to rabbits, 67–68% is excreted, combined with glucuronic acid, whilst with pure *l*-menthol only 48% is excreted. Some 30–40% of the *l*-menthone fed to rabbits is converted to hydroxy compounds, which are excreted combined with glucuronic acid.

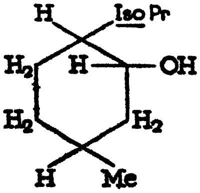
*d*-*Iso***menthone.** *d*-*Iso*menthone also undergoes reduction in the rabbit, since *d*-*iso*menthylglucuronide is excreted in the urine (Williams, 1940). The other possible reduction product, *d*-*neoiso*menthol, is not excreted. It is interesting to compare this biological reduction with the chemical reduction in acid solution by hydrogen in the presence of a platinum catalyst; the product is *d*-*neoiso*menthol, no trace of *d*-*iso*-menthol being formed (Hückel and Niggemeyer, 1939).



*iso*menthone



*iso*menthol

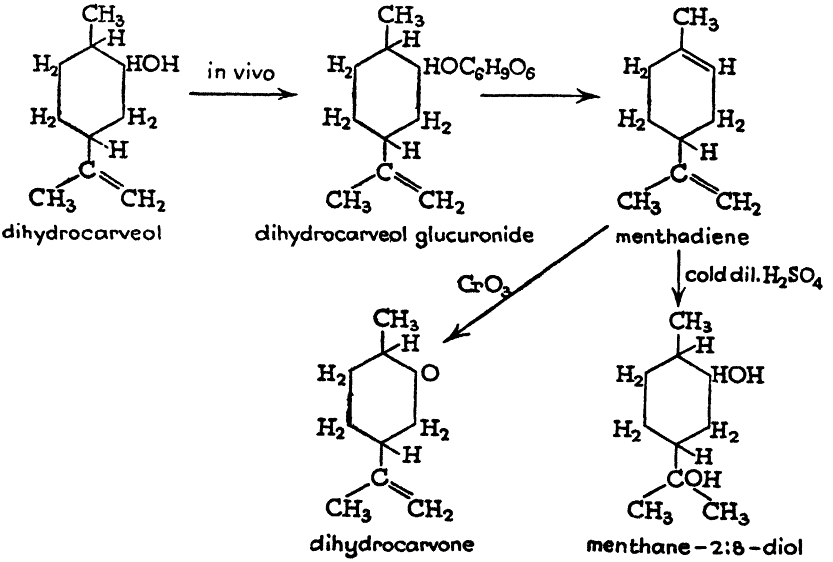


*neoiso*menthol

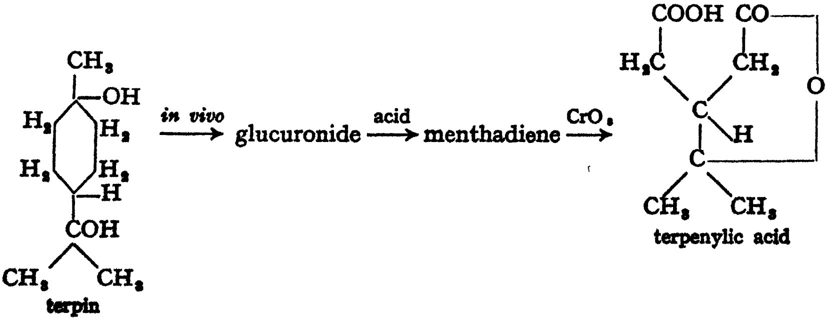
## MONOCYCLIC ALCOHOLS

In general, the monocyclic terpene alcohols conjugate with glucuronic acid in the animal body, the glucuronic acid residue being attached to the existing OH group of the terpene. The fate of that fraction of the terpene alcohol not excreted as a glucuronide is obscure and there is no information as to whether the monocyclic ring in these compounds undergoes fission *in vivo*.

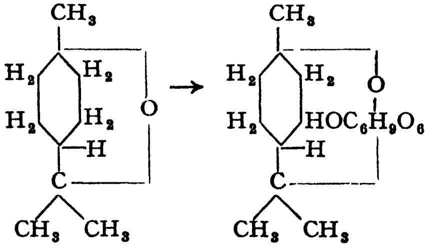
**Dihydrocarveol** is excreted by the rabbit as dihydrocarveol glucuronide (Hämäläinen, 1912). Hydrolysis of the glucuronide with dilute sulphuric acid results in the formation of a menthadiene which on hydration with cold dilute $H_2SO_4$ forms *p*-menthane:2:8-diol, and on oxidation with chromic oxide gives dihydrocarvone.



**Terpin.** Hämäläinen also showed that the dihydric alcohol terpin (menthane-1:8-diol) forms a glucuronide giving a menthadiene on acid hydrolysis. The menthadiene gives terpenylic acid on oxidation with chromic oxide and a mixture of terpin hydrate and terpineol on hydration with sulphuric acid.



The anhydride of terpin, **cineole**, apparently undergoes oxidation *in vivo* with the formation of hydroxycineole which is excreted as hydroxycineoleglucuronic acid. This alcohol may be 2- or 3-hydroxycineole.



cineole 3-hydroxycineoleglucuronide

## THE MENTHOLS

*l*-**Menthol** or mint camphor is a compound of great importance in medicine as an anaesthetic and antiseptic; it is a constituent of numerous common remedies. It is the chief component of the oil of peppermint and itself has a strong odour of peppermint. Its isomerides *d*-menthol, the *d*- and *l*-*iso*-, -*neo*- and -*neoiso*-menthols are not known to occur naturally.

Of the three optical isomerides, *d*-, *dl*-, and *l*-menthol, the latter is the most toxic to white rats, the order of toxicity and lethal action being *l*- > *dl*- > *d*- (Hazard and Lardé, 1936). *l*-Menthol is more active physiologically than *d*-, and liquid menthol, which is a mixture of equal parts of the three isomerides, is the most potent local anaesthetic (Macht, 1939). As will be seen below, the menthols also differ quantitatively in the extent to which they conjugate with glucuronic acid in the animal.

It is well known that *l*-menthol conjugates in the rabbit forming *l*-menthyl-β-*d*-glucuronide, a compound which is an important source of glucuronic acid as it is easily isolated from the urine of rabbits receiving menthol. About half the *l*-menthol fed to rabbits is excreted combined with glucuronic acid (Williams, 1938*a*); the fate of the other half has not been investigated, but it is possible that ring fission occurs with considerable degradation of the menthol molecule. In dogs a considerable oxidation of menthol takes place and only about 5% of the *l*-menthol fed can be recovered in the urine as menthyglucuronide. According to Quick (1924) the percentage of a dose of *l*-menthol which is excreted combined with glucuronic acid in the rabbit depends on the magnitude of the dose. The larger the dose the less of it is conjugated, showing that the rabbit has a limited capacity for conjugating menthol with glucuronic acid (see Table 22).

The extent to which a given dose of menthol is conjugated with glucuronic acid also depends on the isomeride fed, since Williams (1938*a*, 1940) has shown that menthanols of the *dextro* configuration are excreted combined with glucuronic acid to a greater extent than the *l*-menthanols (see Table 23). This fact has been used by Williams (1939; 1940*a*) for the optical resolution of *dl*-menthol and *dl*-*iso*menthol.

### TABLE 22

*Relation of Dose of* l-*Menthol to Excretion of Glucuronic Acid*
(*after Quick,* 1924)

| Rabbit 1 | | Rabbit 2 | |
|---|---|---|---|
| Dose g. | Percentage conjugated | Dose g. | Percentage conjugated |
| 0·5 | 48·0 | 1·0 | 46·0 |
| 1·0 | 41·3 | 2·0 | 47·5 |
| 2·0 | 42·3 | 3·0 | 40·7 |
| 2·5 | 37·9 | 3·5 | 34·9 |
| 3·0 | 37·1 | 4·0 | 26·1 |
| 3·5 | 36·6 | 4·5 | 19·0 |
| — | — | 5·0 | 16·9 |

### TABLE 23

*Conjugation of Various Isomeric Menthanols with Glucuronic Acid in the Rabbit* (*dose* 1 *g./kg.*)

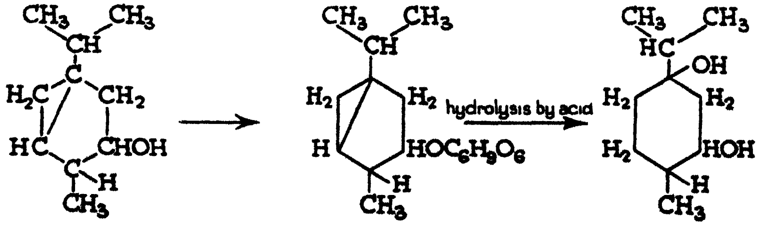
| *l*-menthanols | Percentage conjugated | *dl*-menthanols | Percentage conjugated | *d*-menthanols | Percentage conjugated |
|---|---|---|---|---|---|
| *l*-menthol | 48 | *dl*-menthol | 59 | d-menthol | 70 |
| *l*-*iso*menthol | 45 | *dl*-*iso*menthol | 55 | *d*-*iso*menthol | 65 |
| | | | | *d*-*neo*menthol | 67·5 |

## BICYCLIC TERPENES

Terpenes containing bicyclic structures are fairly stable *in vivo,* and only in a few cases are they degraded to monocyclic structures or open-chain compounds.

### (1) The Thujane or Sabinane Structure

Saturated sabinane compounds are degraded to monocyclic structures in the body. Thus **thujyl alcohol** ($[\alpha]_D$ + 64°) [1] when fed to rabbits is excreted as *p*-menthane-2:4-diolglucuronide and thujylglucuronide.



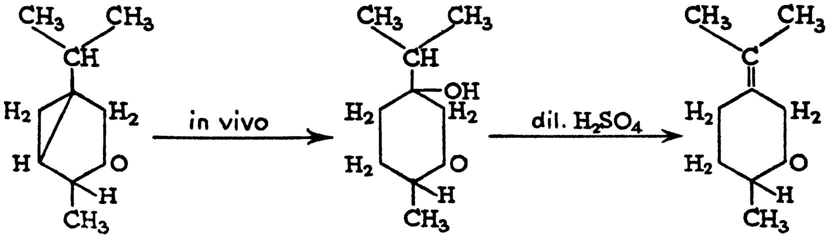
**thujyl alcohol** **thujylglucuronide** ***p*-menthane-2:4-diol**

---

[1] Rotation of actual material fed.

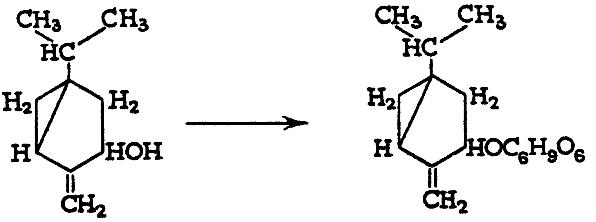
The former glucuronide on hydrolysis with acid yields *p*-menthane-2:4-diol.

**Thujone** ($[\alpha]_D + 76°$) behaves similarly, forming a glucuronide which is probably that of *p*-menthane-2-one-4-ol since the glucuronide on hydrolysis with dilute $H_2SO_4$ gives the same product, carvenone, as obtained on dehydration of *p*-menthane-2-one-4-ol.



thujone *p*-menthane-2-one-4-ol carvenone

When, however, the sabinane structure contains an unsaturated link, the tendency is for the bicyclic structure to survive, since **sabinol** when fed to animals yields sabinylglucuronide (m.p. 82–83°) in which the bicyclic structure survives (Hämäläinen, 1912; 1912*a*; cf. also Fromm and Clemens, 1903).

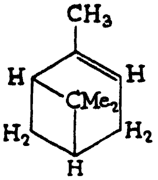


sabinol sabinylglucuronide

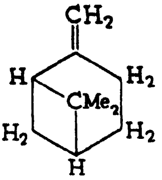
## (2) **The Pinane Structure**

**α-Pinene** is the main constituent of turpentine oil. The earliest record of its fate in the animal body is in a paper published in 1876 by Baumann, who found that when dogs were rubbed with turpentine oil, there was a definite increase in the output of ethereal sulphates. One can interpret this result as meaning that a phenolic substance was formed and excreted conjugated with sulphuric acid. This result also indicates that, like *cyclo*hexylcarboxylic acid, the pinene structure may be dehydrogenated *in vivo* to an aromatic ring.

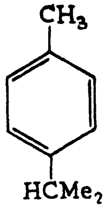
Nothing definite is known as to whether the α- and β-pinenes are converted into monocyclic structures *in vivo*. Hämäläinen has shown that both α- and β-pinene are converted in the rabbit into conjugated glucuronides (of *p*-menthanetriol ?) of undetermined nature, and these glucuronides on heating with dilute acids give the aromatic hydrocarbon cymene.



α-pinene



β-pinene



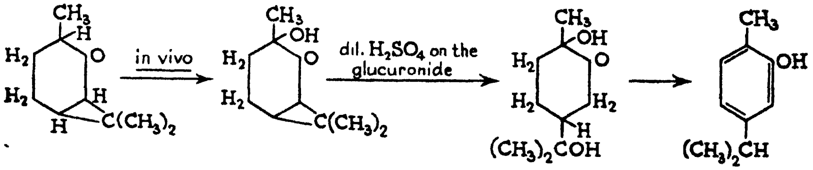
cymene

When administered to sheep, α-pinene causes gross albuminuria and has a toxic action on the liver and kidneys (Harvey, 1942).

### (3) The Carane Structure

This structure contains a propylene ring occurring as a bridge in a six-carbon ring (cf. the thujane structure). Judging from the case of carone, the only compound of this group whose fate *in vivo* has been studied, the bicyclic structure is not degraded to a monocyclic compound.

**Carone.** According to Rimini (1909), carone is oxidised in the dog to 4-hydroxycarone, which is excreted conjugated with glucuronic acid. On hydrolysing a solution of the glucuronide with dilute sulphuric acid the phenol, carvacrol, is obtained. 3-Ketoterpin is probably also formed during the hydrolysis.

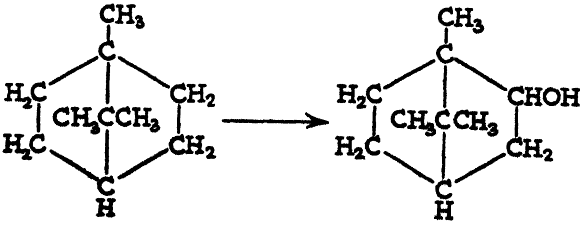


carone 4-hydroxycarone 3-Ketoterpin carvacrol

### (4) The Camphane Structure

The camphane structure is apparently fairly stable *in vivo* and degradation to monocyclic structures occurs only to a small extent. Most compounds of the bicyclic terpene type, whose fate in the animal body has been studied, contain the camphane nucleus, and it is to this group that common camphor belongs.

**Camphane.** When camphane is fed to an animal it is oxidised to a mixture of *d*- and *l*-borneols which are excreted as the corresponding glucuronides (Hämäläinen, 1912).



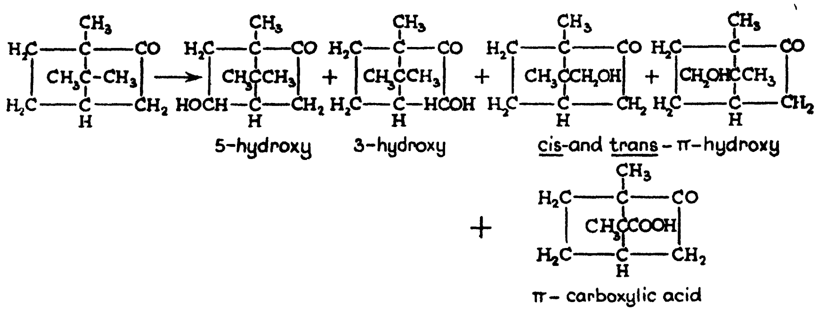
camphane borneol

***d*-Borneol** is the main constituent of Borneo camphor and its metabolism in the animal body is a fairly straightforward process. In man it is almost entirely excreted as bornylglucuronide for about 80% of a 2 g. dose is excreted conjugated in 10 hours (Pryde and Williams, 1936). In the dog some 52% is excreted as the glucuronide (Quick, 1928*b*). According to Magnus-Levy (1907) *d*- and *l*-borneol are equally well conjugated in dogs and rabbits, but the work of Pryde and Williams (1934) suggests that *d*-borneol is excreted by the dog as a glucuronide to a greater extent than is the *l*-isomeride. In the rabbit, 86% of *d*-bornyl acetate is excreted as the glucuronide, 92% of *l*-bornyl acetate and 95% of *dl*-borneol (Hämäläinen, 1909; Schüller, 1911; Hämäläinen and Sjöström, 1910). The glucuronides of the borneols and of the ***iso*borneols** form crystalline zinc salts whose insolubility in water make them useful substances for isolating the bornylglucuronides (cf. Fromm and Clemens, 1901).

## The Metabolism of the Camphors

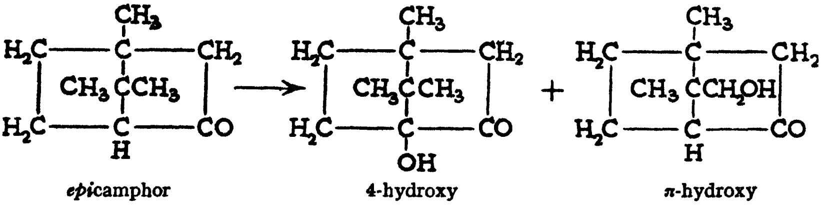
**Common camphor** (*d*-camphor) has had considerable use in medicine as a temporary cardiac stimulant and therefore its fate in the animal body has been the subject of many investigations. In 1879 Schmiedeberg and Meyer isolated three camphor metabolites from dog urine; these were "α-camphoglycuronic acid" ($[\alpha]_D - 32{\cdot}85°$), "β-camphoglycuronic acid", and a nitrogenous compound which they called "uramidocamphoglycuronic acid". The α-compound was obtained in largest amount and all three gave hydroxycamphor (campherol) on hydrolysis. Schmiedeberg and Meyer used common or *dextro*-camphor, and later work has shown that the fate of *dl*- and *l*-camphor in the body is similar to that of the *dextro*-isomeride. Little definite information regarding the constitution of these hydroxycamphors was obtained for nearly fifty years until the problem was investigated by Asahina and Ishidate (1933, 1934, 1935). These workers showed that the α-camphoglycuronic acid of Schmiedeberg and Meyer contained two campherols which they identified as 3- and 5-hydroxycamphors. They also demonstrated that α- and β-camphoglycuronic acid only differed in the proportion of the 3- and 5-hydroxycamphors they contained. Further investigation showed that when very large amounts of biosynthetic campherols are worked up, other metabolic products occur in small amounts. These are *cis*- and *trans*-π-hydroxycamphors and *trans*-π-apocamphor-7-carboxylic acid. The main oxidation product of camphor is 5-hydroxycamphor, with appreciable amounts of the 3-hydroxy derivative. The metabolism of camphor as far as oxidation is concerned may be summarised as shown on p. 180.

The formulae below show that the biological oxidation of camphor takes place mainly in a position *para* to the keto group with some oxidation in the *ortho* position. Despite the fact that oxidation in the π-position occurs only to a small extent, it proceeds further than that in the 3- and 5-positions, for a small amount of the π-carboxylic acid can be isolated. It should be noted that the keto group of camphor suffers no change and is therefore biochemically unlike aromatic and aliphatic keto groups.

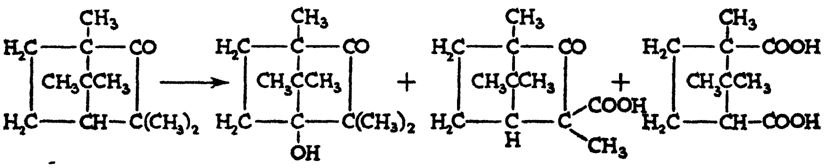


A number of other camphors have also been studied and the results show that the position at which the camphor ring is oxidised *in vivo* depends on the position of substituents.

In the case of *epi***camphor**, the position of the keto group is 3 and not 2 as in camphor itself and whereas a small amount of oxidation takes place in the π-position, the main oxidation takes place in the 4-position. The main product of *epi*camphor oxidation in the dog is 4-hydroxy*epi*camphor with small amounts of π-hydroxy*epi*camphor (Reinartz, Zanke and Schaefers, 1934). In this case the main oxidation takes place *ortho* to the keto group.



When **3:3-dimethylcamphor** is fed to dogs three products can be isolated from the urine, namely 4-hydroxy-3:3-dimethylcamphor, 3-methylcamphor-3-carboxylic acid and camphoric acid.

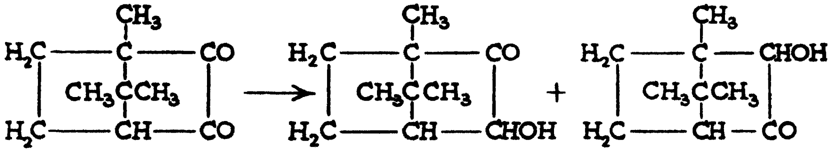


In 3:3-dimethylcamphor, both the fenchane and the camphane structures are discernible, but oxidation takes place in positions similar to those which are oxidised biologically in fenchone (see p. 183), i.e. 4 and π and not 3, 5 and π as in camphor.

*l*-**Camphor** and *dl*-**camphor** are also converted to campherols in the animal body (Mayer, 1908; Magnus-Levy, 1906).

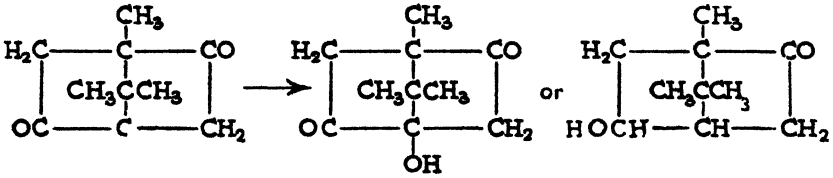
**The Ketocamphors.** The fate of two ketocamphors has been investigated, namely **camphorquinone** and **diketocamphane** (5-oxocamphor). These two substances correspond to the *o*- and *p*-quinones of the aromatic series. Their behaviour in the organism is quite different, since with the *ortho* compound, camphoquinone, both keto groups are reducible *in vivo*, whereas in diketocamphane neither keto group is attacked and oxidation takes place elsewhere in the molecule (Reinartz, Zanke and Faust, 1934).

When camphorquinone is fed to dogs, glucuronides are excreted which on hydrolysis give 3-hydroxycamphor and 2-hydroxy*epi*camphor. Here we find that either keto group may be reduced but not both simultaneously.



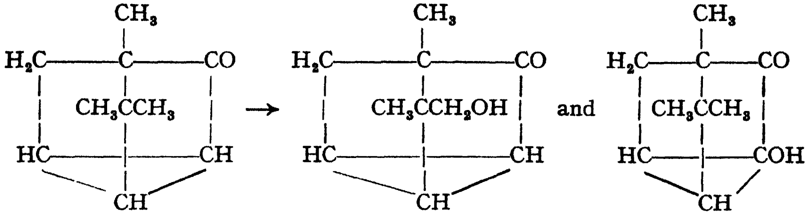
camphorquinone 3-hydroxycamphor 2-hydroxy*epi*camphor

According to Reinartz *et al.* (1934) neither keto group in 5-oxo**camphor** is reduced, but oxidation takes place in the 4-position to form 4-hydroxydiketocamphane and an acid, $C_{10}H_{12}O_3$, which they believe to be *β-cyclo*camphanone-*π*-carboxylic acid (*β-cycloiso*ketopinic acid). The formation of the latter compound is a surprising reaction, since it involves reduction at $C_5$, followed by dehydration at $C_3$ and $C_5$ and simultaneous oxidation at $C_\pi$. The fate of 5-oxocamphor has been reinvestigated by Ishidate, Kawahata and Nakazawa (1941), who were unable to confirm the results of Reinartz *et al.* They found that on feeding *d*-5-oxocamphor to dogs and then hydrolysing the conjugated glucuronide excreted, the only substance produced was *d*-5-hydroxycamphor. The formation of this compound involves the reduction of one keto group.



diketocamphane 4-hydroxydiketocamphane 5-hydroxycamphor

*Cyclo***camphanone** is a tricyclic camphor which can be conveniently mentioned at this point. Reinartz *et al.* (1934) have shown that in the dog this compound is mainly oxidised in the *π*-position. It will be recalled that in most other camphors little oxidation takes place in the *π*-position. Some oxidation of *cyclo*camphanone with the formation of a tertiary hydroxyl in the 3-position does take place. However, neither degradation to a bicyclic or a monocyclic structure nor reduction of the keto group occurs during its metabolism.



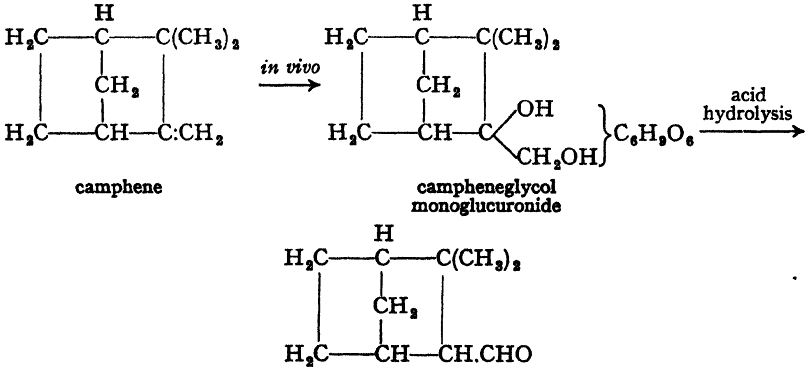
*cyclo*camphanone

*cis*- and *trans*- *π*-hydroxy*cyclo*camphanone

3-hydroxy*cyclo*camphanone

## (5) The Isocamphane Structure

The hydrocarbon **camphene** is interesting since it forms a glycol in the rabbit. The compound excreted is campheneglycol*mono*glucuronide which was isolated as a laevorotatory potassium salt (Fromm, Hildebrandt and Clemens, 1902–3). It is not known to which hydroxyl group the glucuronic acid is attached. On hydrolysis with acid the compound breaks up to glucuronic acid and camphene glycol which itself undergoes further change to camphenilaldehyde.

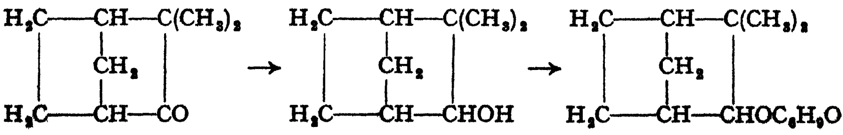


**camphene**

**campheneglycol monoglucuronide**

**camphenilanealdehyde**

**Camphenilol** ($[\alpha]_D + 8{\cdot}6°$) conjugates without change in the rabbit and is excreted as camphenilolglucuronide, m.p. 150–153° (Hämäläinen, 1912). The corresponding camphor, **camphenilone,** is unlike most camphors of the bicyclic terpenes for its keto group is reduced to a secondary alcohol group. The inactive form was fed by Hämäläinen and a mixture of *d*- and *l*-camphenilols was produced. The glucuronide excreted contained more *d*- than *l*-camphenilol.

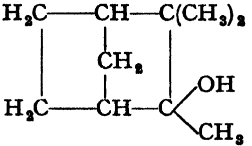


**camphenilone**

**camphenilol**

**camphenilolglucuronide**

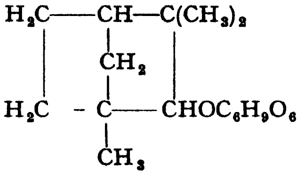
*d*- and *l*-**Isoborneols** are mainly excreted by animals as *iso*bornylglucuronides, the glucuronic acid being attached to the tertiary hydroxyl group (Hämäläinen, 1909).



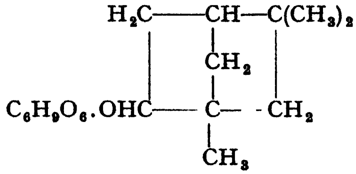
*iso*borneol

### (6) The Fenchane Structure

The fenchane structure appears to be stable *in vivo*. Both *l*-**fenchyl** ($[\alpha]_D$ — 13·6°) and *l*-*iso***fenchyl alcohols** ($[\alpha]_D$ — 24·7°) are excreted in rabbits as the corresponding glucuronides (Hämäläinen, 1912).

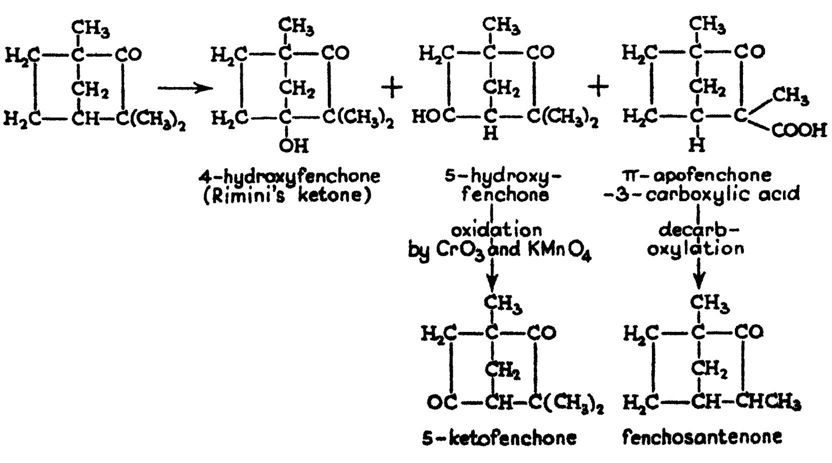


*l*-fenchylglucuronide



*l*-*iso*fenchylglucuronide

*d*-**Fenchone.** The fate of a camphor of this group, namely, *d*-fenchone, which occurs in fennel oil has been studied by Rimini (1901, 1909) and by Reinartz and Zanke (1936). Rimini's work showed that in the dog fenchone was probably oxidised to 4-hydroxyfenchone. Reinartz and Zanke showed that there were other products as well as Rimini's ketone. The latter workers separated, as lead salts, the glucuronides from the urine of dogs receiving *d*-fenchone. The lead was removed by sulphuric acid and the resulting solution hydrolysed. In the mixture of hydroxyfenchones which resulted, the presence of 4- and 5-hydroxyfenchones and $\pi$-apofenchone-3-carboxylic acid was demonstrated. The hydrolysate, as such, could not be separated into its constituents, but on oxidation



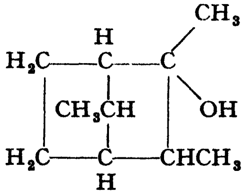
with $CrO_3$ and $KMnO_4$, it yielded unchanged 4-hydroxyfenchone, whilst the 5-hydroxy derivative was recovered as 5-ketofenchone. The carboxylic acid was identified by the fact that on heating above its melting point it was decarboxylated to fenchosantenone. (See p. 183).

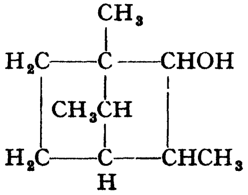
### (7) **Derivatives of Santene**

Santene is present in East India sandalwood oil. Its metabolism has not been investigated, although the fates of some of its derivatives have been studied (Hämäläinen, 1912).

**β-Santenol** or santene hydrate (inactive) is converted in the rabbit to β-santenolglucuronide, which on hydrolysis yields β-santene.

**α-Santenol** (inactive) behaves similarly forming α-santenolglucuronide. The corresponding ketone, **α-santenone** or π-norcamphor, probably behaves in a similar manner to camphor *in vivo*, for the keto group survives and oxidation takes place elsewhere in the molecule, α-santenonolglucuronide being excreted. However, the location of the hydroxyl in this biosynthetic α-santenonol is uncertain.


β-santenol

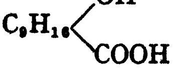

α-santenol

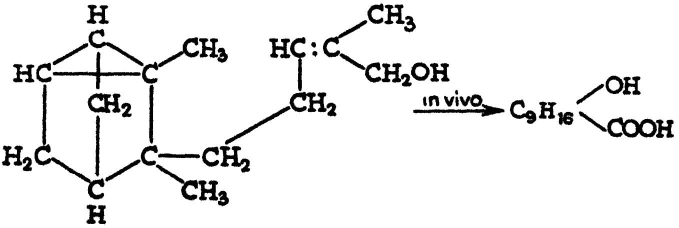
## SESQUITERPENES

Little is known regarding the fate of the sesquiterpenes in the animal body. In the rabbit the open-chain sesquiterpene alcohol, **farnesol,** is probably oxidised to the corresponding acid, farnesic acid (Fischer and Bielig, 1940).

$$(CH_3)_2C{:}CHCH_2CH_2C(CH_3){:}CHCH_2CH_2C(CH_3){:}CHCH_2OH$$
farnesol

The tricyclic sesquiterpene alcohol, **α-santalol,** undergoes considerable change *in vivo* (Hildebrandt, 1902) for an isoprene residue is eliminated, a methyl group is oxidised to —COOH and an acid of the formula

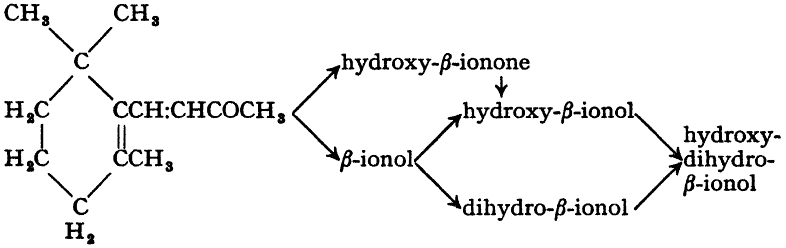

$C_9H_{16}\begin{cases} OH \\ COOH \end{cases}$ is excreted.



α-santalo

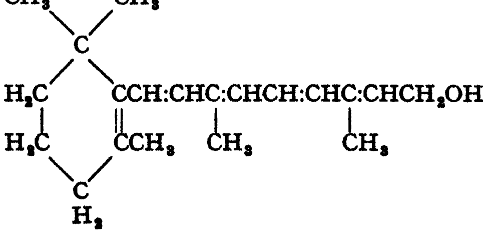
## IONONE

The α- and β-ionones are synthetic ketones possessing the odour of violets and are isomerides of irone, the odoriferous principle of the iris root and probably of violets. The special interest of ionone in metabolism lies in the fact that vitamin A (axerophtherol) (see formula below) contains the ring structure which is present in β-ionone. Little is known regarding the fate of vitamin A in the body, but the fate of orally administered **β-ionone** in the rabbit has been studied by Bielig and Hayasida (1940). Several metabolic products are excreted, indicating a complex series of changes. The products isolated from the urine and identified are, unchanged β-ionone, β-ionol, dihydro-β-ionol, hydroxy-β-ionol, hydroxydihydro-β-ionol and hydroxy-β-ionone. Tetrahydro derivatives and dehydrogenation products are not found. A study of the action of ozone on hydroxy-β-ionone shows that oxidation of one of the ring methyls occurs. From these facts it can be deduced that (1) the —CO group in the side-chain is reduced to —CHOH, (2) one double bond, but not both, is hydrogenated and this double bond is probably the one in the side-chain, (3) oxidation of one of the ring —$CH_3$ groups to —$CH_2OH$ occurs. Traces of unidentified aldehydes and acids are also found, indicating oxidation of a $CH_3$ group through —$CH_2OH$ and —CHO to —COOH. The metabolic fate of β-ionone can probably be represented by the following scheme:—



β-ionone

It is obvious that the primary products are β-ionol and hydroxy-β-ionone, the former produced by a reduction of the 4-carbon side-chain and the latter by oxidation of one of the ring methyls.



vitamin A

**Phytol** or phytyl alcohol contains a structure which is found in a number of biologically important substances, e.g. chlorophyll and vitamin

$K_1$ (2-methyl-3-phytyl-1:4-naphthoquinone). It appears probable that phytol is oxidised in the rabbit to phytic acid[1] (2:6:10:14-tetramethyl-$\Delta^1$-pentadecene-1-carboxylic acid (cf. farnesol). An acid which may be phytic acid was isolated from the urine of rabbits receiving phytol by injection (Fischer and Bielig, 1940).

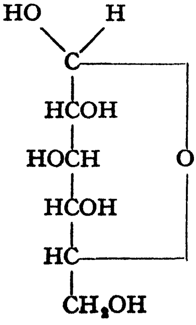
$$(CH_3)_2CH(CH_2)_3CHCH_3(CH_2)_3CHCH_3(CH_2)_3CCH_3{:}CHCH_2OH$$

phytol

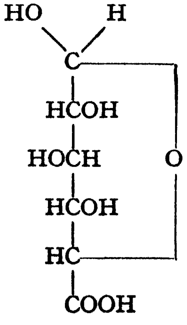
## THE ORIGIN OF THE GLUCURONIC ACID OF CONJUGATED GLUCURONIC ACIDS

It seems appropriate in this chapter to discuss the origin in the body of the glucuronic acid utilised in the conjugation process because in many of the investigations on this problem terpenes have been used as glucuronidogenic substances. The terpenes, with perhaps a few exceptions, are excreted combined with glucuronic acid and with no other conjugating agent, and the extent of conjugation may be as high as 95% of the dose (cf. borneol). Furthermore, glucuronic acid was first discovered as a component of a metabolite of camphor.

Chemically *d*-glucuronic acid is an oxidation product of *d*-glucose, and on this ground it appears probable that it is derived in the body from a carbohydrate precursor. This relationship has provided a working hypothesis and many studies on the origin of glucuronic acid have been attempts to prove or disprove that it is derived from glucose. However, this chemical relationship can be misleading for some of the earlier workers went as far as to state, without any supporting experimental evidence, that glucuronic acid was produced in the body by direct oxidation of glucose. Such a simple and straightforward explanation is not in keeping with the balance of experimental evidence.



*β-d*-glucose



*β-d*-glucuronic acid

**The Intermediary Glucoside Hypothesis.** The earliest hypotheses concerning the origin of glucuronic acid were those put forward by Schmiedeberg and Meyer (1879), by Sundvik (1886) and by Fischer and

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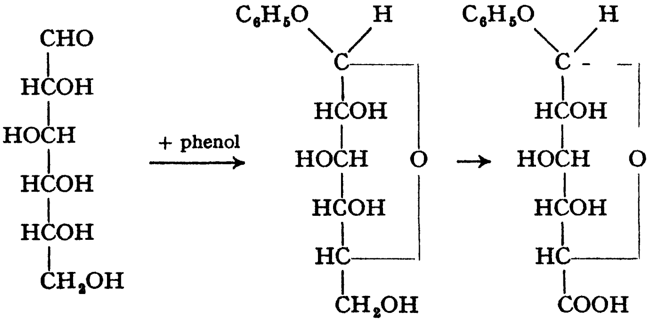
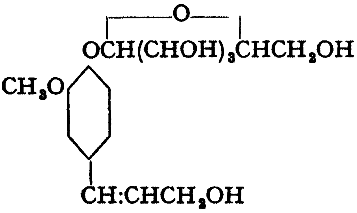
[1] This acid must not be confused with inositolhexaphosphoric acid, which is also known as phytic acid.

Piloty (1891). In essence, these hypotheses are very similar. Schmiedeberg and Meyer, having decided that glucuronic acid was a derivative of glucose, concluded that it must, therefore, be a normal intermediate product in the combustion of glucose in the body, but by combining with a camphor derivative it escaped complete decomposition. The hypotheses of Sundvik and of Fischer and Piloty are identical and are less vague than the speculations of Schmiedeberg and Meyer. The Sundvik-Fischer-Piloty hypothesis postulates glucoside formation as an intermediate in the synthesis of conjugated glucuronic acid. It envisages compounds such as phenol, camphor, etc., first combining with glucose to form a glucoside. The easily oxidised aldehyde group of glucose is thus protected from oxidation, which now takes place at the primary alcohol group with the formation of a conjugated glucuronic acid, thus :—

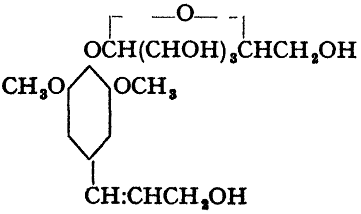


phenylglucoside phenylglucuronide

Experiments have been carried out by Hildebrandt (1905, 1909), the results of which have been interpreted as supporting the glucoside hypothesis. On subcutaneous injection of coniferin (coniferylglucoside), syringin (methoxyconiferylglucoside) or bornylglucoside into rabbits, the corresponding conjugated glucuronic acids are excreted in the urine. This observation has alternative explanations. Either the glucoside is directly oxidised *in vivo* to the glucuronide or it is first hydrolysed to give the aglucone which then combines with preformed glucuronic acid or its precursors. The latter explanation is favoured by the following facts :—



coniferin



syringin

(*a*) The glucoside phloridzin when administered to animals is excreted as phloridzinglucuronide (Schuller, 1911), in which the original glucose molecule is unchanged and glucuronic acid is attached to one of the originally free hydroxyl groups of phloridzin (see p. 78). In this case glucuronic acid is not derived from the glucose of phloridzin.

(*b*) When phenyl-β-glucoside is injected into or fed to rabbits there is an increased excretion of ethereal sulphate (Pryde and Williams, 1936), which is the same as that produced by an equivalent dose of phenol, thus indicating the *in vivo* hydrolysis of phenylglucoside to phenol.

(*c*) Neither bornyl-β-glucoside nor phenyl-β-glucoside gives rise to conjugated glucuronic acids when added to the perfusing blood in a pump-lung-liver-kidney preparation which could synthesise conjugated glucuronic acid from free borneol or phenol (Hemingway, Pryde and Williams, 1934).

(*d*) Guinea-pig liver slices, which can produce conjugated glucuronic acid in the presence of suitable concentrations of borneol, do not produce glucuronide from bornyl-β-glucoside (Lipschitz and Bueding, 1939).

The results of the above experiments obviously dispose of the Sundvik-Fischer-Piloty hypothesis.

### The Three-Carbon Compound Hypothesis

Other hypotheses have been put forward which suggest proteins, glycogenetic amino acids, glycogen and the three carbon metabolites of carbohydrate metabolism as sources of glucuronic acid. From what follows, it will be seen that any of these substances may act as sources of glucuronic acid because they are converted during normal metabolism into the precursor or precursors of glucuronic acid.

The earlier experiments on the origin of glucuronic acid using starving, depancreatised or phloridzinised animals gave results which were extremely contradictory. One reason for this was probably the lack of a satisfactory method for the quantitative estimation of glucuronic acid in biological fluids.

**Experiments with Starving Animals.** The early work of Thierfelder (1886) suggested a definite protein origin for glucuronic acid. He starved a dog for 17 to 21 days, after which time the animal was considered free from glycogen. The animal was then given 6 g. of chloral hydrate and it excreted 5·72 g. of urochloralic acid. From this result Thierfelder claimed that the glycogen-free animal could synthesise carbohydrate for the purposes of detoxication, the protein of the body being the probable source of carbohydrate. In starving rabbits, Mayer (1902) found that their ability to synthesise conjugated glucuronic acid in response to administered camphor was diminished, but the normal output could be restored by administration of glucose, hence he claimed that glucuronic acid originated from the glucose of the body. The experiments of Meyer could not be confirmed by von Fenyvessy (1909), who found that rabbits fasted for twelve days showed neither decrease in glucuronic acid output following phenol, camphor or chloral hydrate nor increase when fed these substances plus glucose. The experiments of Mandel and Jackson (1903) on starving dogs seemed to confirm Thierfelder's suggestion. They fed starving dogs on camphor and investigated the nitrogen output under various conditions. Their findings were that on feeding large amounts of glucose to the camphor-treated dogs, there was a large diminution in both the nitrogen and the camphoglycuronic acid outputs, whereas on feeding chopped meat there was a corresponding increase in both. Constant elimination of camphoglycuronic acid occurred as long as meat and camphor were fed.

Experiments on fasting animals, however, are liable to uncertainty, for there is no criterion whereby it can be decided whether an animal is free from glycogen. Concerning such experiments, Quick (1926*a*) makes the following remark: " In view of our present knowledge concerning the tenacity with which the body conserves its store of glycogen and its power to convert protein to glucose and glycogen, these experiments prove nothing one way or the other." In order to overcome this difficulty, Schmid (1936) experimented with hibernating frogs. The frog can produce conjugated glucuronic acid in response to glucuronidogenic compounds. During the period of hibernation, its total glycogen is of the order of 0·25 mg., whereas a well-fed frog has 60–200 mg. of glycogen in its liver. Schmid kept three groups of frogs in jars with 50 c.c. of water. The first group was well fed, the second starved and the third starved but their water contained glucose or glycogen. Each animal then received 15–20 mg. of menthol orally. In the first and third groups there resulted no sign of intoxication, and the surrounding water eventually gave a positive Tollens naphthoresorcinol test for glucuronic acid. The second group fell into narcosis within one hour of the administration of menthol and died in 1–2 days ; the surrounding water gave a negative Tollens reaction. These experiments indicate that in the frog, at least, preformed carbohydrates are necessary for the formation of conjugated glucuronic acid.

None of the above experiments on fasting animals is conclusive, but they strongly suggest that glucuronic acid may be derived from both carbohydrate and protein sources.

### Experiments with Phloridzinised and Depancreatised Animals

Experiments on the origin of glucuronic acid using phloridzinised animals were made by Loewi (1902), who assumed that glucose and glucuronic acid have the same precursor. On this assumption it follows that the administration of a glucuronidogenic drug to a diabetic animal should cause a decrease in glucose excretion which would correspond to the glucuronic acid produced. Loewi used phloridzinised dogs which had been fasted for two weeks, and found after giving camphor neither decrease in sugar excretion nor a greater drop than expected in the nitrogen excretion, so that the glucose-nitrogen ratio was raised rather than diminished. The conclusions were therefore reached that glucose and glucuronic acid had different precursors and that glucuronic acid was not derived from glucose. However, Loewi's results are insecurely based, for he was handicapped by the lack of a satisfactory method for the determination of conjugated glucuronic acid and relied on polarimetry.

The latter method is obviously unsatisfactory, for the urine in these experiments would contain at least four optically active substances, namely, glucose, camphoglycuronic acid, phloridzin and phloridzin-glucuronide. The last substance was not isolated until 1911 (see p. 79).

Working on the same idea as Loewi, Quick (1926*a*) tackled the problem with depancreatised dogs, using sodium benzoate and borneol as glucuronidogenic drugs. Quick measured the $\frac{\text{glucose}}{\text{nitrogen}}$ ratio, and, after the glucuronidogenic drug, the $\frac{\text{glucose} + \text{glucuronic acid}}{\text{nitrogen}}$ ratio, in these animals. If glucose and glucuronic acid have the same precursor, the numerical values of these ratios in the depancreatised dog should be the same. This was found to be approximately true; Quick's results are quoted in Table 24.

TABLE 24

*The Glucose/Nitrogen Ratio of Depancreatised Dogs given Sodium Benzoate and Borneol* (*after Quick*, 1926*a*)

Dog 10. Weight 8·2 kg Operated April 10th. No insulin or food given after April 13th

| Date | Sugar, $D$ gm. | Nitrogen, $N$ gm. | Glucuronic acid expressed as glucose, $G$ gm. | $D/N$ | $\frac{D+G}{N}$ | Drug fed |
|---|---|---|---|---|---|---|
| April 15 | 24·0 | 3·6 | — | 6·65 | — | |
| 16 | 6·65 | 2·69 | — | 2·47 | — | |
| 17 | 4·55 | 2 68 | — | 1·70 | — | |
| 18 | 0·94 | 3·67 | 4·35 | 0·26 | 1·44 | 5 g. sodium benzoate |
| 19 | 11·8 | 5·88 | — | 2·01 | — | |
| 20 | 14·85 | 6·66 | — | 2·22 | — | 200 g meat |
| 25 | 8·6 | 2 96 | — | 2·90 | — | |
| 26 | 7·55 | 2·91 | — | 2·60 | — | |
| 27 | 4·65 | 2·56 | 1·52 | 1·82 | 2·41 | 3 g. borneol |
| 28 | 6·16 | 2·58 | — | 2·39 | — | |
| 29 | 7·08 | 3·02 | — | 2·35 | — | |

Quick concluded that glucuronic acid and glucose produced in the fasting diabetic dog have the same precursor, possibly lactic acid, and that whenever there is a demand on the organism for glucuronic acid it is produced at the expense of the potential glucose. Since the production of glucuronic acid was not accompanied by an increase of urinary nitrogen (see Table 24), it appeared that the animal did not have to catabolise additional protein, but could synthesise glucuronic acid from the protein it had to break down for its maintenance as a fasting depancreatised animal. Thus it appears from Quick's experiments that, in the fasting diabetic animal, glucuronic acid can be derived from protein presumably *via* glucogenetic amino acids.

**Experiments on Normal Animals.** Quick (1926*a*) showed that glucuronic acid could also be derived from carbohydrates, not necessarily glucose. Since glucose and glucuronic acid may arise from a common precusor in the diabetic organism, it is pertinent to ask whether a normal animal can produce glucuronic acid from carbohydrates or whether it must, like the diabetic organism, synthesise it from protein. Normal dogs given sufficient carbohydrate can produce conjugated glucuronic acid in response to ingested borneol or sodium benzoate without causing a marked increase in urinary nitrogen. This indicates that the organism can synthesise glucuronic acid from carbohydrates. However, after a period of fasting, glucose administered with sodium benzoate does not prevent an increased nitrogen catabolism, so it appears that glucose cannot be used directly. Quick, therefore, concludes that the precursor of glucuronic acid is derived more readily from glycogen and glycogenetic amino acids than from glucose. This view is supported by the experiments of Lipschitz and Bueding, using the tissue slice technique (see below).

The suggestion that glycogen may be used in the synthesis of glucuronic acid, receives direct support from the experiments of Dziewiatkowski and Lewis (1944). These workers found that four hours after the administration of *l*-menthol or sodium tertiary butyl acetate to well-fed young rats, the glycogen content of their livers is much depleted compared with that of control animals receiving sodium *n*-butyrate (which does not form a glucuronide) or water (see Table 25). Both *l*-menthol and sodium tertiary butyl acetate cause the excretion of extra glucuronic acid in considerable amounts.

TABLE 25

*Glycogen Content of Rat Livers after the Administration of* l-*Menthol and Sodium Tertiary Butyl Acetate*

| Substance fed | Average liver glycogen % |
|---|---|
| Water | 3·58 |
| *l*-menthol | 1·60 |
| Sodium tertiary butyl acetate | 0·95 |
| Sodium *n*-butyrate | 6·39 |

**Experiments with Tissue Slices.** Quick (1926*a*) suggested that glucuronic acid might be derived from a simple compound such as lactic acid, which in the diabetic animal is derived from protein and in the normal animal from the degradation of glucose. This suggestion has received considerable support from the tissue slice experiments of Lipschitz and Bueding (1939). These workers found that in the guinea-pig, the liver was the main tissue concerned in the synthesis of glucuronic acid in the presence of borneol; the process requires oxygen for it is abolished by anaerobiosis and is catalysed by a heavy metal for it is inhibited by cyanide (0·001 *M* KCN). The synthesis is also inhibited by sodium fluoride and monoiodoacetate, indicating the participation of phosphorylation in the synthesis. Liver slices poor in carbohydrate, i.e.

from fasted animals, produce very little glucuronic acid in the presence of borneol, but the synthesis is greatly stimulated by the presence of certain three-carbon compounds such as dihydroxyacetone, lactate and pyruvate Other biologically important carbohydrate metabolites such as glyceraldehyde, phosphoglycerate, methylglyoxal, α-glycerophosphate and hexosediphosphate are ineffective. Glucuronic acid itself cannot be utilised by the liver slice for the synthesis of conjugated glucuronic acid, and glycogen, glucose, glucosamine and fructose are only slightly effective.

It appears, therefore, that glucuronic acid for conjugation purposes is derived neither from preformed glucuronic acid[1] nor directly from glucose, glycogen or protein, but from a simple three-carbon precursor (or precursors). It can, however, be derived indirectly from glucose, glycogen and the glucogenetic portion of proteins insofar as they can be converted during their metabolism into the simple precursor or precursors of glucuronic acid as it is found in the conjugated glucuronic acids.

The question of whether or not preformed glucuronic acid can combine directly with hydroxy compounds is one which deserves more careful investigation. The occurrence of the enzyme β-glucuronidase in tissues presupposes the existence of the following reversible reaction :—

$$\beta\text{-glucuronide} \rightleftarrows \text{aglucone} + \text{glucuronic acid}$$

There is ample evidence to show that glucuronidase can catalyse this reaction to the right, but very little to show that the enzyme can carry out the synthesis of glucuronide except that of Houet, Dutchateau and Florkin (1941), who demonstrated a slight synthesis of conjugated glucuronic acid from glucuronic acid and borneol by glucuronidase at *p*H 4·5. The work of De Meio and Arnoldt (1944) also suggests that phenol may combine directly with glucuronic acid on liver slices. The conjugation of phenol in tissue slices is inhibited by 0·002 *M* monoiodoacetate (cf. Lipschitz and Bueding, 1939), but on addition of glucuronate the conjugation can be re-established. Lactate is ineffective in re-establishing the conjugation. Glucuronic acid, however, does not re-establish conjugation after inhibition by cyanide or under anaerobic conditions. The inhibitory effect of monoiodoacetate appears from this, to affect the formation of glucuronic acid and not its conjugation with phenol.

The exact nature of the precursor or precursors of glucuronic acid and the mechanism of the synthesis of conjugated glucuronic acid have still to be worked out.

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[1] Administered glucuronic acid is only metabolised with difficulty in man and the rabbit (Biberfeld, 1914; Quick, 1928*b*) and is largely excreted unchanged. Some, however, may be transformed into furan-2:5-dicarboxylic acid in man, for Flaschenträger, Cagianut and Meier (1945) found that after the administration of 5 g. of *d*-glucurone or *d*-galacturonic acid, the normal output of furandicarboxylic acid in urine was increased 5–15 times.

## TABLE 26

*Biosynthetic Glucuronides of the Terpene Group*

| *β-d*-Glucuronide | M.P. | Specific rotation $[\alpha]_D$ in alcohol | Reference |
|---|---|---|---|
| *l*-menthyl | 110°, 94° | − 110° | e.g. Fromm and Clemens (1901), Williams (1939), Fischer (1910) |
| *d*-menthyl | 120–122° | + 6·4° | Williams (1939) |
| *d*-*iso*menthyl | 126° | − 43·2° | ,, (1938*a*) |
| *dl*-*iso*menthyl | — | − 56° ($NH_4$ salt in water) | ,, (1939*a*) |
| *d*-*neo*menthyl | 145° | − 15° | ,, (1940) |
| *p*-menthane-2-one-4-ol | — | + 11·8° (K salt in water) | Hämäläinen (1912) |
| *p*-menthane-3:8-diol (3-monoglucuronide) | 192° | $-15{\cdot}2 \xrightarrow{20\text{hr.}} -21{\cdot}7^\circ$ | Kuhn and Löw (1938) |
| *d*-bornyl | 164–165° (anhydr.)<br>96–97° (hydrate) | − 37° | Hämäläinen (1909) |
| *l*-bornyl | 162–163° (anhydr.)<br>96–97° (hydrate) | − 69° | ,, ,, |
| *dl*-bornyl | 94–95° (hydrate) | − 48° | ,, ,, |
| *dl*-*iso*bornyl | 104–106° (hydrate) | − 42·6° | ,, ,, |
| *l*-fenchyl | 124–126° | − 63° (aq. acetone) | ,, (1912) |
| *l*-*iso*fenchyl | 140–145° | − 81° ($H_2O$) | ,, ,, |
| camphenilol | 150–153° | − 63° ($H_2O$) | ,, ,, |
| α-santenol | 160–161° | − 56·6° ($H_2O$) | ,, ,, |

## *Chapter Twelve*

# The Metabolism of Heterocyclic Compounds

COMPOUNDS containing heterocyclic rings have been found in nature which are of considerable biological importance; thus we have nicotinic acid, vitamins $B_1$, $B_2$, $B_6$ and E, tryptophan and the porphyrins, to mention only a few. In addition, many synthetic heterocyclic compounds are drugs of great value and include such compounds as antipyrine and its allies, the barbiturates, sulphathiazole, phenothiazine, etc.

A large number of heterocyclic compounds exhibit aromatic characteristics and such compounds as furan, thiophene, pyrrole, pyridine, etc., and their derivatives whilst possessing their own individuality, behave chemically in an analogous manner to benzene and its derivatives. This behaviour is also maintained to a notable degree when heterocyclic compounds are metabolised in the animal organism, although in a few instances they undergo changes which are more or less specific. These latter changes include, for example, methylation of pyridine compounds and the synthesis of furfuracrylic acid from furfural.

The following list of biochemical reactions illustrates the similarity between the heterocyclic and carbocyclic series:—

(*a*) Pyridine, antipyrine and phenothiazine are oxidised *in vivo* to phenolic compounds and cause an increased output of ethereal sulphates.

(*b*) Furan-, thiophene- and pyridine-carboxylic acids are excreted partly combined with glycine, forming compounds analogous with hippuric acid. In the hen, furancarboxylic acid forms a conjugate with ornithine; this is not the case with thiophene- and pyridine-carboxylic acids.

(*c*) Furfural and thiophene aldehyde are oxidised *in vivo* to the corresponding carboxylic acids and are thus similar to benzaldehyde.

(*d*) Methylpyridines are oxidised to the corresponding carboxylic acids, a process analogous with the oxidation of toluene to benzoic acid.

(*e*) Furanpropionic acid is oxidised to pyromucic acid thus behaving analogously with phenylpropionic acid.

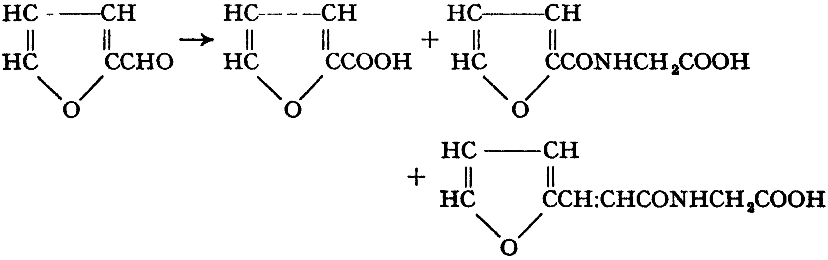
(*f*) Amino groups attached to heterocyclic ring are often acetylated *in vivo*, for pyramidone results in the excretion in man of 4-acetylaminoantipyrine.

(*g*) Many heterocyclic hydroxy compounds form glucuronides.

The main metabolic changes of heterocyclic compounds are therefore the same as those undergone by carbocyclic compounds, but in one or two instances they differ. Methylation is a process, which, although it occurs as a normal reaction in the formation of creatine and certain other N-methyl derivatives in the body, is quite uncommon amongst the carbocyclic compounds, although it is a general *in vivo* reaction for pyridine and quinoline derivatives.[1] The biological formation of furfuracrylic acid from furfural is apparently specific for this aldehyde and does not occur even with the closely related thiophene aldehyde. The metabolism of pyrrole is somewhat obscure, but as far as we know it does not fall into line with furan, thiophene and pyridine, for it does not form a phenolic compound and its carboxylic acid appears not to form a glycine conjugate.

## THE METABOLISM OF FURAN[2] AND ITS DERIVATIVES

Newly discovered chemical analogies between furan and benzene led Jaffe and his co-workers to investigate the fate of furan compounds in the animal body. The fate of benzene *in vivo* had been studied many years earlier and it was known that it underwent oxidation to phenol. Jaffe and Cohn (1887) fed **furfural** to dogs and rabbits and found that none of it was excreted unchanged. The products isolated were pyromucic acid, pyromucuric acid (furoylglycine) and furfuracryluric acid (furylacryloylglycine).



Pyromucuric acid was isolated from rabbit urine as a salt with mineral bases, but from dog urine it was obtained combined with urea presumably as a salt, the formula of which was given as $C_7H_7NO_4,CO(NH_2)_2$.

The furylacrylic acid derivative is of special interest since it appears that furfural is the only aromatic aldehyde which undergoes this synthesis. The glycine conjugate is analogous with cinnamoylglycine (see p. 113).

The formation of furylacrylic acid is believed by Jaffe and Cohn to be the result of a Perkin synthesis involving acetic acid and is analogous

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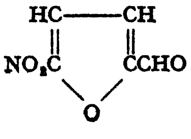
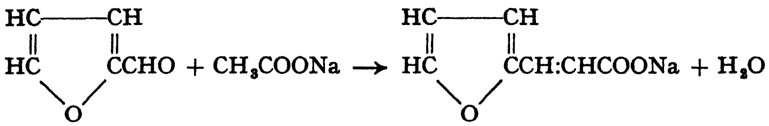
[1] It appears probable that tellurium and tellurium compounds are converted in the animal into dimethyl telluride, $Te(CH_3)_2$ (Hofmeister, 1893–4). Selenium may also be methylated in the body.

[2] The 5-nitro derivatives of furfural, furyl alcohol and furoic acid are bacteriostatic *in vitro* (Dodd and Stillman, 1944), e.g. :—

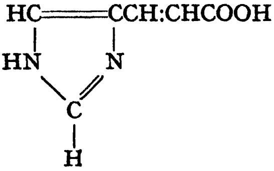


with the chemical synthesis of cinnamic acid from sodium acetate and benzaldehyde :—

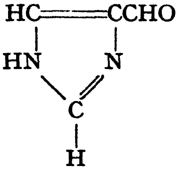
$$C_6H_5CHO + CH_3COONa \rightarrow C_6H_5CH{:}CHCOONa + H_2O$$



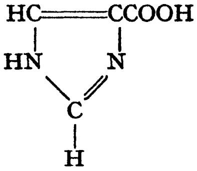
A number of aromatic aldehydes have been tested to see if they undergo a Perkin synthesis *in vivo*, but not one has been found to do so (Friedmann and Turk, 1913). **Urocanic acid** [1] is found in the urine of dogs after large doses of histidine (Kotaki and Konishi, 1922), and contains an acrylic acid group and is actually iminazoleacrylic acid.



urocanic acid



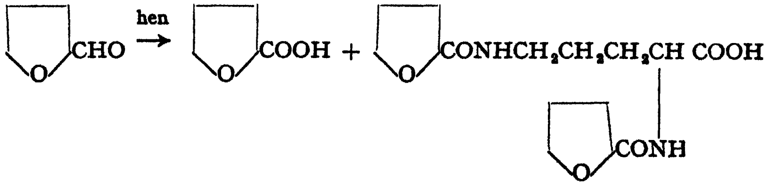
glyoxaline formaldehyde



glyoxaline carboxylic acid

In order to test whether urocanic acid could be formed by a Perkin synthesis in an analogous manner to furylacrylic acid, Barger and Dakin (1916) fed **glyoxaline formaldehyde** to a dog. No excretion of urocanic acid was observed and small amounts of glyoxaline carboxylic acid were excreted.

In the hen (Jaffe and Levy, 1888) furfural does not form an acrylic acid derivative or a glycine conjugate and the main products of its metabolism are pyromucic acid and pyromucinornithuric acid (difuroylornithine) :—



Furfuracryluric acid is also produced when **furfuracrylic acid** itself or **furylpropionic acid** is fed (Friedmann, 1911*a*; Sasaki, 1910). Furylpropionic acid is excreted to the extent of 21·5% as furfuracryluric acid and 18·4% as pyromucuric acid:—

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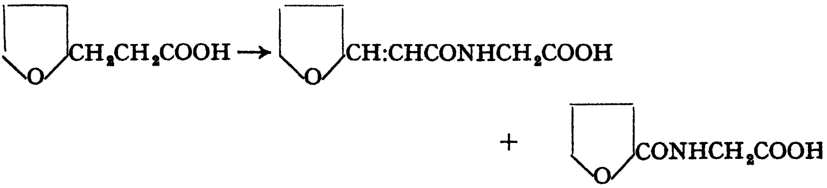
[1] Originally discovered in urine of two dogs (Jaffe, 1874*a*; 1875; Siegfried, 1898) in which it appeared to be an abnormal but constant constituent.



In this case the acrylic acid derivative is probably formed by direct desaturation of furylpropionic acid. Furfuracrylic acid itself on subcutaneous injection into dogs (Friedmann) is converted to the extent of 29·4% into furfuracryluric acid, 22·4% into pyromucic acid which is apparently excreted unconjugated and 0·1% into acetofuran. **Furoylacetic acid** is mainly excreted as pyromucuric acid together with small amounts of acetofuran. These reactions may be summarised as follow:—

$$\begin{tikzcd}
 & C_4H_3O\cdot CH{:}CHCONHCH_2COOH \\
C_4H_3O\cdot CH{:}\,CHCOOH \arrow[ur] \arrow[r] \arrow[dr] & C_4H_3O\cdot COOH \\
 & C_4H_3O\cdot COCH_3
\end{tikzcd}$$

$$\begin{tikzcd}
C_4H_3O\cdot COCH_2COOH \arrow[r] \arrow[dr] & C_4H_3O\cdot CONHCH_2COOH \\
 & C_4H_3O\cdot COCH_3
\end{tikzcd}$$

Dakin (1922*b*) points out that the detection of acetofuran as a metabolic product in the metabolism of furfuracrylic acid indicates that furoylacetic acid is an intermediate in its metabolism. The above facts when taken together indicate that the metabolism of furylpropionic acid may follow the following route to pyromucic acid:—

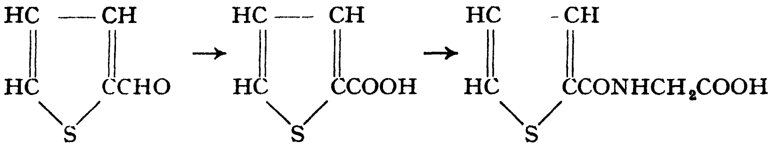
$$C_4H_3O.CH_2CH_2COOH \rightarrow C_4H_3O.CH{:}CHCOOH \rightarrow$$
$$C_4H_3O.C(OH){:}CHCOOH \rightarrow C_4H_3O.COCH_2COOH \rightarrow C_4H_3O.COOH$$

and they appear to support the view of Carter (1941) that unsaturated acids are obligatory intermediates in the metabolism of fatty acids.

## THIOPHENE AND ITS DERIVATIVES

**Thiophene** when fed to dogs behaves like a typical aromatic hydrocarbon. It is oxidised and increases the output of ethereal sulphates (Heffter, 1886). According to Chilcote (1945) thiophene when administered orally to rabbits does not increase the ethereal sulphate output, but there is an increase in the urinary neutral sulphur corresponding to 50% of the thiophene ingested. This indicates that a thienylmercapturic acid is formed although it was not isolated. About 25% of the extra organic sulphur excreted was in a form which yielded thiophene when the urine was acidified and heated. **2-Bromothiophene** behaves like thiophene in the rabbit (Chilcote, 1945). **Thiophene α-carboxylic acid**

behaves like benzoic acid and is excreted by rabbits as a glycine conjugate, α-thiophenuric acid (α-thiophenoylglycine, m.p. 171–172°) (Jaffe and Cohn, 1888). **α-Thiophenealdehyde** is mainly excreted by dogs and rabbits as α-thiophenuric acid. No trace of free thiophene carboxylic acid is excreted and the aldehyde does not form an acrylic acid derivative (Cohn, 1894*a*).



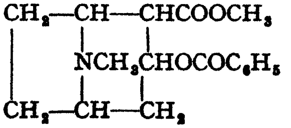
## PYRROLE AND ITS DERIVATIVES

**Pyrrole** behaves chemically more like phenol than like benzene, but it is easily destroyed *in vivo* and does not increase the output of ethereal sulphates. **Pyrrole-α-carboxylic acid** is said to be excreted free and not conjugated with glycine (Ginsberg, 1890). Pyrrole is fairly toxic to animals, but the α-carboxylic is almost non-toxic (Ginsberg).

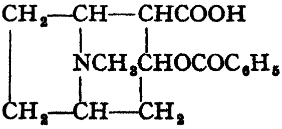
## COMPOUNDS CONTAINING REDUCED PYRROLE RINGS

**Cocaine and Related Compounds.** Reduced pyrrole rings occur in the important drugs, **cocaine, atropine** and **nicotine.** The first two substances contain, as a basic nucleus, two condensed pyrrolidine rings with a —CH.$NCH_3$.CH— group in common. Little is known as yet regarding the nature of their metabolic products.

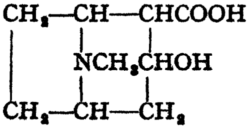
**Cocaine** is to a small extent excreted unchanged in the urine, the amount eliminated in mice, rats, guinea-pigs, cats and dogs depending upon the *p*H of the urine. With a markedly acid urine, as much as 16% of the injected dose may be excreted unchanged (Oelkers and Vincke, 1935). More *l*-**cocaine** is excreted unchanged by cats than *d*-**cocaine** or *d-pseudo***cocaine**, and this fact may indicate that the *d*-isomerides are degraded to a greater extent than the *l*-forms (Gruhn, 1925). It has been suggested by Langecker and Lewit (1938) that cocaine is converted in the rabbit first to benzoylecgonine by the loss of a methyl group and then to ecgonine by loss of the benzoyl group. In this way cocaine may be detoxicated for both these products are less toxic than cocaine.



cocaine

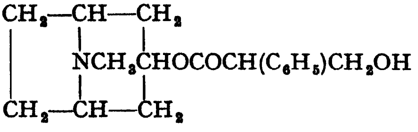


benzoylecgonine

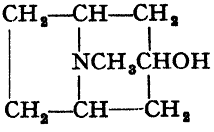


ecgonine

**Atropine** (α-phenyl-β-hydroxypropionic ester of tropine) is probably hydrolysed, and thereby inactivated, to tropine in animals (Langecker and Lewit, 1938), but sound experimental evidence on this point is lacking.



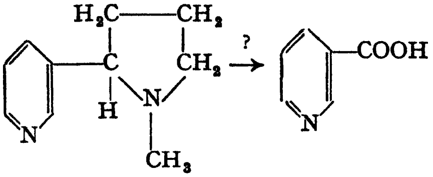
atropine



tropine

**Nicotine.** The fate of this alkaloid in the body should be of great interest, especially in view of the fact that users of tobacco absorb small amounts into their systems. Nicotine contains both the pyridine and the reduced pyrrole ring structures. The metabolism of the pyrrolidine ring as such is unknown, although it is possible that it is mostly destroyed as in the case of piperidine. There is some evidence based on our knowledge of the metabolism of nicotine for this latter suggestion.

When small doses (0·2 mg./kg.) of nicotine are injected subcutaneously into dogs some 10% is excreted unchanged in the urine and the rest is, in part, present in the nicotinic acid fraction of the urine (Haag and Larson, 1942). Nicotine is not demethylated to nornicotine (Larson and Haag, 1943).



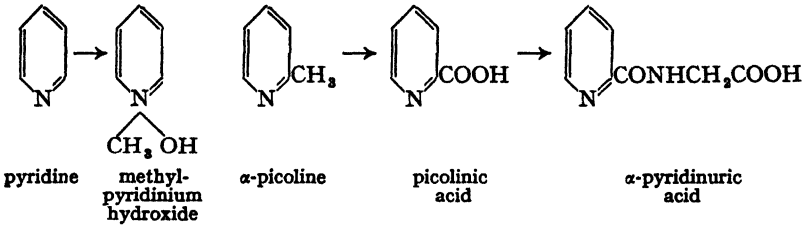
nicotine nicotinic acid

If nicotinic acid is excreted, then its carboxyl group must be derived from the pyrrolidine ring indicating the destruction *in vivo* of the N-methylpyrrolidine residue. Haag and Larson were unable to find in the urine conjugated products of nicotinic acid, such as methyl pyridinium hydroxide carboxylic acid, nicotinuric acid and trigonelline, in measurable amounts (see p. 201).

## PYRIDINE AND ITS DERIVATIVES

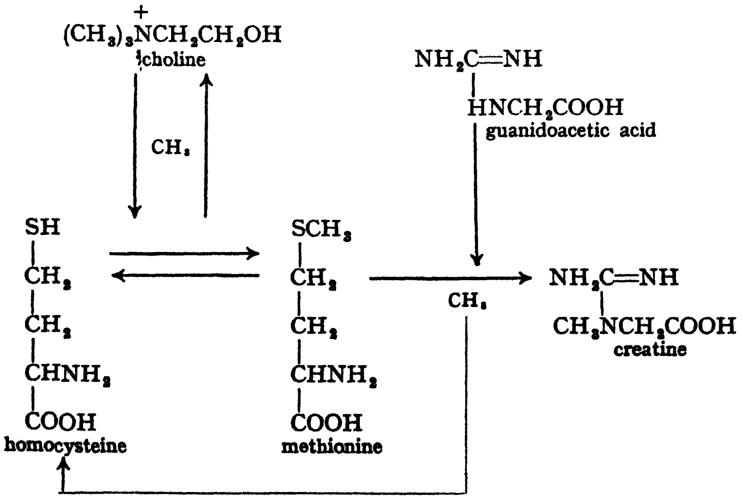
The metabolism of **pyridine** was first investigated by His (1887), who discovered that it was oxidised and excreted as an ethereal sulphate and a conjugated glucuronide. His identified the biological oxidation product of pyridine as methylpyridinium hydroxide, the molecule having undergone both oxidation and methylation. **Piperidine,** the reduced form of pyridine, is not methylated (Novello, Harrow and Sherwin, 1926). The work of His was confirmed by Cohn (1894*a*), who also discovered that

**α-picoline** (α-methylpyridine) was oxidised *in vivo* to picolinic acid and excreted as the glycine conjugate, α-pyridinuric acid :—



A large number of pyridine derivatives have been shown to undergo methylation in the animal body. The dog (Abderhalden, Brahm and Schittenhelm, 1909), the pig, the goat (Totani and Hoshiai, 1910), the hen (Hoshiai, 1909), the rat and man are able to methylate pyridine derivatives. According to Abderhalden and Brahm (1909), the rabbit, unlike other animals, does not methylate pyridine.

The recent work of du Vigneaud and his colleagues (1941) has thrown much light on methylation processes in the animal body. According to this school, methylations which normally occur in the body such as the synthesis of creatine from guanidoacetic acid are carried out by the transfer of methyl groups (transmethylation) from such compounds as methionine, choline and betaine. The transfer of methyl groups in the synthesis of creatine may occur according to the following scheme (du Vigneaud) :—



The ultimate source of methyl groups is probably dietary methionine,[1] choline and betaine and the possibility of "methyl deficiency" can therefore be envisaged. Whether the methylation of pyridine is carried out by the same systems and is ultimately dependent on dietary sources of methyl groups has yet to be ascertained. If, however, this turns out to be true, then some detoxication processes will be dependent upon an ample supply of methyl groups in the diet. The inability of the rabbit to methylate pyridine (but see p. 202), whereas it presumably can methylate guanidoacetic acid to form creatine, is another interesting fact which requires explanation and which may indicate different methylating systems for such compounds as pyridine and guanidoacetic acid (cf. acetylation in the dog, see p. 140).

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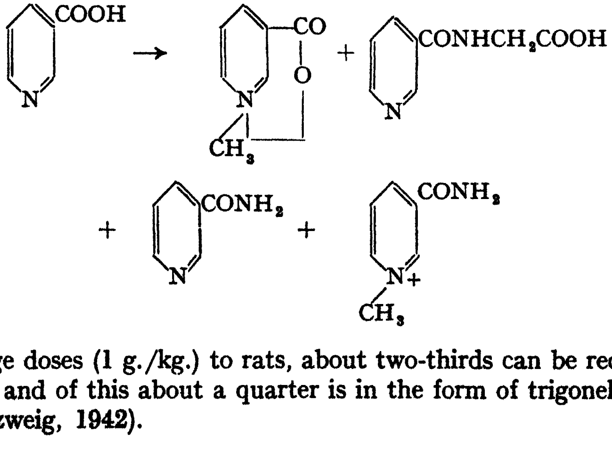
[1] The possibility that cysteine and biological methylation might be connected had also been envisaged by Challenger (1935) who suggested that the biological methylation of arsenic might be preceded by combination with the —SH group of the cysteine component of glutathione. Challenger, however, proposed glycine as the methylating agent involved in biological methylations.

## NICOTINIC ACID AND PYRIDOXINE

These two vitamins are simple derivatives of pyridine and there is now a body of information regarding their metabolism in the animal body.

That **nicotinic acid** is methylated *in vivo* and excreted as trigonelline was first shown by Ackermann (1912). Trigonelline, the anhydride of N-methylpyridinium hydroxide 3-carboxylic acid, is the chief end-product of nicotinic acid metabolism in man, the rat and the dog (Huff and Perlzweig, 1942). The rat excretes some 30% of a 3 mg. dose of nicotinic acid within 24 hours, and 70–80% of that excreted appears in the form of trigonelline, the rest as unchanged nicotinic acid, nicotinuric acid and nicotinamide. In addition to this, small amounts of $N^1$-methylnicotinamide are also excreted. This latter substance may be identical with the fluorescent material[1] $F_2$ described by a number of workers occurring in normal human urine in small amounts, but in large amounts after administration of nicotinic acid or its amide (Najjar and Wood, 1940; Najjar and Holt, 1941, 1941*a*; Huff and Perlzweig, 1943). The metabolism of nicotinic acid can therefore be represented as follows:—



After large doses (1 g./kg.) to rats, about two-thirds can be recovered in the urine and of this about a quarter is in the form of trigonelline (Huff and Perlzweig, 1942).

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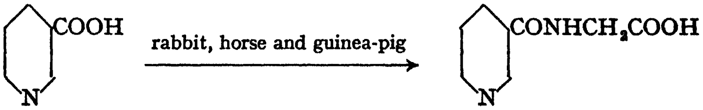
[1] **For a discussion on the nature of the fluorescent material $F_2$ see O'Brien (1944).**

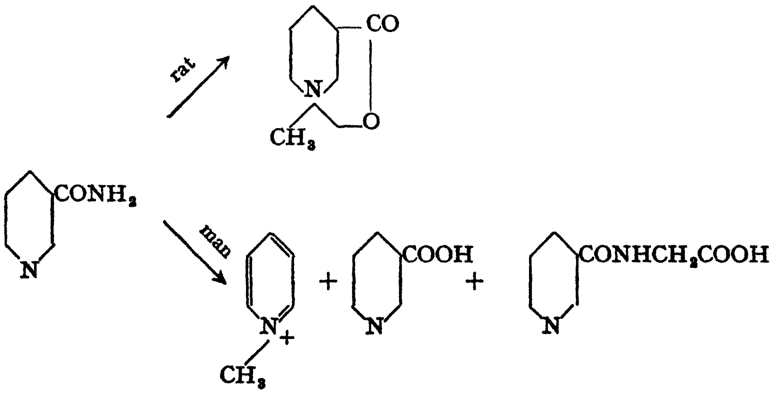
The rabbit, the horse and the guinea-pig do not excrete nicotinic acid as methylated derivatives, for neither trigonelline nor N¹-methylnicotinamide are found in the urine of these animals when they are fed nicotinic acid (Komori and Sendyu, 1926; Sarett, 1943; Handler, 1944; Pearson and Luecke, 1945). The acid is mainly excreted as nicotinuric acid :—



The horse destroys orally administered N¹-methylnicotinamide and Huff, Pearson and Perlzweig (1946) make the interesting suggestion that the horse and the rabbit may be able to methylate nicotinic acid and its derivatives like other animals, but that the methylated derivatives are rapidly destroyed as soon as they are formed.

**Nicotinamide** in 1·5 mg. doses is excreted by the rat to the extent of 30% in 24 hours, and the excreted material is largely trigonelline, with small amounts of unchanged amide (Huff and Perlzweig, 1942). In man, nicotinamide is methylated and excreted as N¹-methylnicotinamide, for Huff and Perlzweig (1943) gave two subjects a 3 g. dose of nicotinamide and in the urine collected during the following three days they found 900 mg. of the N¹-methyl derivative, 120 mg. of which was isolated.

Large doses of nicotinamide also result in the excretion by man of appreciable amounts of nicotinuric acid, but with small doses (50 mg.) none of the glycine conjugated is found and the excreted material is mainly N¹-methylnicotinamide with small amounts of nicotinic acid and the unchanged amide (Johnson, Hamilton and Mitchell, 1945).



Rat liver slices can synthesise *in vitro* N¹-methylnicotinamide from methionine and nicotinamide (Perlzweig, Bernheim and Bernheim, 1943).

**Trigonelline** is excreted to a large extent unchanged by man, the dog and the rabbit. In the rat 20–40% of a 1·5 mg. dose of trigonelline is excreted unchanged, a proportion which is much less than in man, the dog or the rabbit (Huff and Perlzweig, 1942). The fate of the rest of the trigonelline is at present unknown.

**Nicotinuric acid** is also mainly excreted unchanged by man and the dog, but in the rat a substantial part appears as free nicotinic acid and as trigonelline, and the rat, unlike other species, appears to possess a mechanism for splitting nicotinuric acid.

The rat, unlike man and the dog, is an animal in which the synthesis of nicotinic acid has been definitely demonstrated. This animal on a protein-free diet containing only 7 $\mu$g. of nicotinic acid excretes a daily total of 25–75 $\mu$g. of nicotinic acid derivatives in the urine and 40–90 $\mu$g. in the faeces. This excretion is increased when sources of nitrogen such as casein, amino acids (glycine and *dl*-$\gamma$-amino-*n*-valeric acid) and choline are added to the diet. On withdrawal of these compounds from the diet, the nicotinic acid excretion decreases to its original level. Whilst the total nicotinic acid derivatives excreted is influenced by these compounds, the feeding of choline does not cause an extensive increase in methylated derivatives and glycine does not cause an increase in nicotinuric acid excretion.

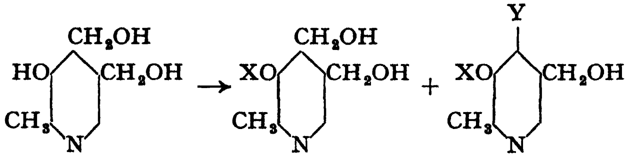
## The Effect of Nicotinamide on Growth

In 1942, it was reported by Stekol that the growth of male rats was inhibited when they were kept on a low protein diet to which 1% nicotinic acid had been added. Further, an independent investigation by Handler and Dann (1942) revealed that nicotinamide but not nicotinic acid depressed the growth of rats of both sexes. Nicotinamide was found to be more highly methylated than nicotinic acid, and this observation was correlated with the difference in the effect of the acid and amide upon growth. Furthermore, the latter workers showed that nicotinamide at lower levels than those required to influence growth was capable of causing a marked fatty infiltration of the liver. Both growth inhibition and fatty livers could be prevented by methionine, choline plus cystine, and choline plus homocystine, but not by cystine, choline or homocystine alone.

We have here a further example of a so-called detoxication process producing a toxic effect by creating a dietary deficiency. Here the deficiency is that of methionine and choline (see Handler and Dann, 1942, for further discussion). The effect of nicotinamide on the growth of rats and in the production of fatty livers in rats is not observed with rabbits and guinea-pigs, for these animals do not, apparently, methylate nicotinamide (Handler, 1944).

**Pyridoxine or Vitamin $B_6$.** Ingested vitamin $B_6$ is excreted by man and the dog in a conjugated form, and at least two such metabolites are known. The rat, on the other hand, does not conjugate vitamin $B_6$ (Scudi, Buhs and Hood, 1942). In addition to the conjugated forms man and the dog also excrete unchanged vitamin $B_6$.

Some evidence regarding the nature of the metabolites of vitamin $B_6$ has been obtained by Scudi, Buhs and Hood (1942). The first metabolite appears to be vitamin $B_6$ conjugated through its phenolic hydroxyl group with glucuronic or sulphuric acid.



1st metabolite

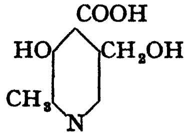
2nd metabolite

X = glucuronic or—$SO_3H$ group

Y = altered hydroxy-methyl group

In the second metabolite, the ring nitrogen is unsubstituted and the 2-methyl group, position 6, and the 5-hydroxymethyl group are unaltered. The 4-hydroxymethyl group is certainly altered and position 3 is conjugated.

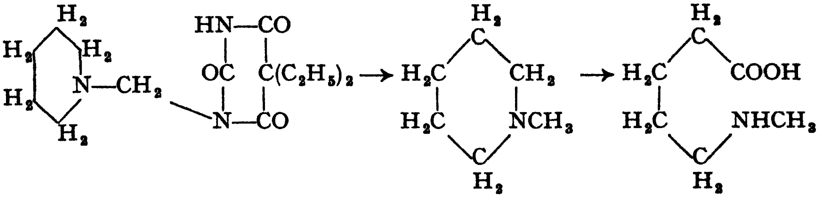
A third metabolite of pyridoxine in man has been isolated from the urine by Huff and Perlzweig (1944). This metabolite has been identified as 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine or 4-pyridoxic acid (m.p. 247–248°) and was isolated from a 24-hour sample of human urine after a dose of 1·25 g. of pyridoxine. The urine was adjusted to *p*H 4·5 with acetic acid and passed through two columns of the zeolite " Decalso ". Pyridoxic acid was obtained from the column by elution with 25% potassium chloride solution. The isolation of this compound shows definitely that the 4-hydroxymethyl group of pyridoxine is oxidised *in vivo.*



4-pyridoxic acid

**Piperidine.** Little is known regarding the fate of piperidine, although Novello, Harrow and Sherwin (1926) have shown that it does not undergo methylation *in vivo.* It is very probable that piperidine is completely oxidised ; there is no evidence to show that it undergoes dehydrogenation to pyridine.

The study of the fate of one derivative of piperidine, **methylene-N-barbital-N-piperidine,** indicates that the piperidine ring is opened. Steudel (1932) showed that on feeding this substance to dogs, small amounts of δ-methylaminovaleric acid could be isolated from the urine. Steudel isolated 1·45 g. of the aurichloride of δ-methylaminovaleric acid after feeding 79·5 g. of the piperidine compound. It appears probable that methylenebarbitalpiperidine is first split into veronal and *N*-methylpiperidine, the latter compound then suffers ring fission.



methylenebarbitalpiperidine N-methylpiperidine δ-methylaminovaleric acid

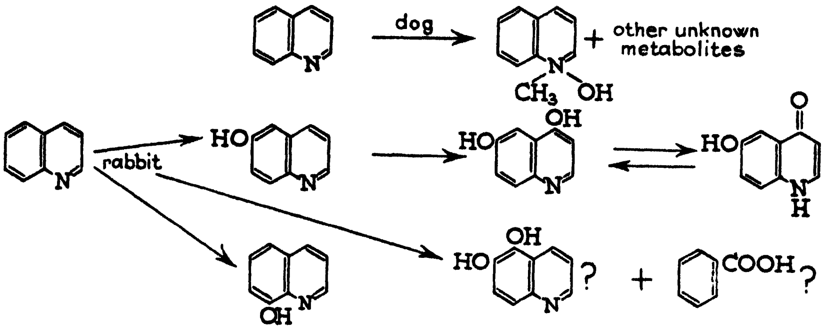
## QUINOLINE AND *ISO*QUINOLINE DERIVATIVES

In quinoline and *iso*quinoline we have two rings, one of which is heterocyclic and the other carbocyclic. In the animal body the heterocyclic ring behaves like pyridine, undergoing oxidation and methylation, and increasing the output of ethereal sulphates and glucuronides. In addition there is a tendency for this ring to form a quinone. The carbocyclic ring behaves as a typical benzene ring and may undergo oxidation to a phenol. There is also a tendency for the carbocyclic ring to be broken up, leaving the free heterocyclic ring to be excreted.

**Quinoline.** In dogs and hens quinoline, like pyridine, is methylated and excreted as methylquinolinium hydroxide. This latter compound is less toxic to dogs, rabbits and hens, and when fed is apparently excreted unchanged (Tamura, 1924). In the rabbit quinoline is not methylated but undergoes oxidation to a variety of products which may be excreted combined with sulphuric and glucuronic acids (Tamura, 1924 ; Novello, Harrow and Sherwin, 1926 ; Fühner, 1906 ; Donath, 1881 ; Scheunemann, 1923). From the urine of rabbits receiving quinoline (20 g.) Scheunemann (1923) isolated, in crystalline form, 6-hydroxy-4-quinolone (176 mg.), 8-hydroxyquinoline (262 mg.) and 6-hydroxyquinoline (343 mg.) There is also excreted a red pigment of unknown constitution, and furthermore Fühner (1906) claims to have isolated 5:6-dihydroxyquinoline. It has also been claimed that a pyridinecarboxylic acid is excreted (Donath, 1881), the occurrence of which would indicate a destruction of the carbocyclic ring, but this was not observed by Scheunemann. He found instead an increased excretion of benzoic acid which appeared as hippuric acid, thus indicating a destruction of the heterocyclic rather than the carbocyclic ring. In the case of atophane (see p. 206) the carbocyclic ring is partly destroyed and Scheunemann thinks that this is not unexpected, for the heterocyclic ring is protected from oxidation by substituents.

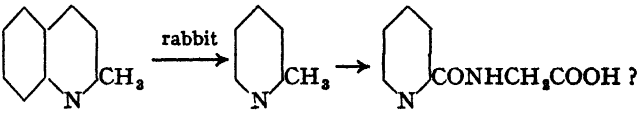
The fate of quinoline in the dog and in the rabbit can be represented as shown on p. 206.

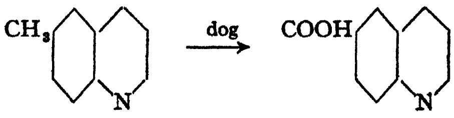
4:6-Dihydroxyquinoline is obviously a further oxidation product of 6-hydroxyquinoline and its formation is reminiscent of the formation of dihydroxybenzenes from benzene *via* phenol.



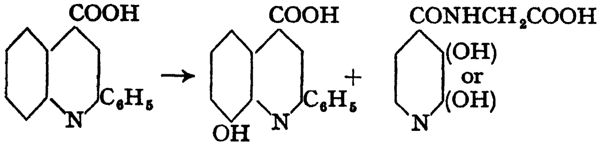
**Hydroxyquinolines.** These phenols behave *in vivo* like any other phenol, and in the dog and rabbit they increase the output of ethereal sulphates and conjugated glucuronides, irrespective of whether the hydroxyl group is in the carbocyclic, as in the case of **8-hydroxyquinoline** (cf. Brahm, 1899), or in the heterocyclic ring as in the case of **4-hydroxyquinoline** (Fenyvessy, 1900). The glucuronide of 8-hydroxyquinoline has been isolated by Brahm by feeding either 8-hydroxyquinoline or its sulphate " quinosol " ; the glucuronide is laevorotatory and its potassium salt shows $[\alpha]_D - 83{\cdot}8°$.

**Methylquinolines. Quinaldine** or **2-methylquinoline** is considerably degraded in the rabbit and is partly excreted as picoline and a compound which is probably the glycine conjugate of picolinic acid (Takahashi, 1930). **6-Methylquinoline,** in which the methyl group is in the carbocyclic ring is said to be converted in dogs to the extent of 7%, based on the amount of material isolated, into the corresponding quinoline-6-carboxylic acid (Cohn, 1895). Cohn was not able to find any metabolites in the urine of dogs and rabbits receiving **8-methylquinoline** which apparently undergoes very extensive degradation.





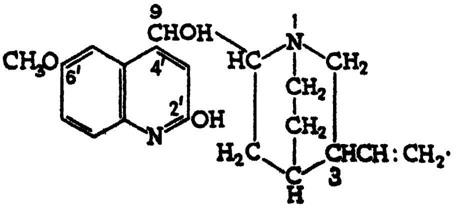
**Atophane** (2-phenylcinchoninic acid) is oxidised in man to 8-hydroxy-2-phenylcinchoninic acid (m.p. 247°). It is also partly degraded, losing its carbocyclic ring and its phenyl side-chain and a hydroxypyridinuric acid (hydroxy*iso*nicotinuric acid) is excreted (Dohrn, 1912). The position of the hydroxyl group in this compound is not known; it could be 2- or 3-hydroxy*iso*nicotinuric acid.



**Quinine** is perhaps one of the most important derivatives of quinoline because of its wide use in the treatment of malaria. Kerner showed as early as 1869 that quinine is partly converted into quinetine *in vivo*, this change involving the oxidation of the vinyl side-chain of the quinuclidine nucleus to —COOH. According to Hertmann and Zila (1918) some 40% of ingested quinine is excreted as such in the urine and faeces, whereas the other 60% undergoes degradation. Few authors have reported degradation products in the urine of patients receiving quinine. Nierenstein (1919*a*) has isolated from the urines of twelve cases of blackwater fever receiving quinine a substance which causes the haemolysis of human and sheep red cells. This substance, haemoquinic acid, can also be isolated from the urine of quinine-treated malaria cases and is probably 6-methoxyquinoline-4-ketocarboxylic acid. The presence of quinetine in urine after ingestion of quinine has been denied by several workers, but Nierenstein (1919) was able to isolate it together with unchanged quinine from the urine of a normal subject in the first 2 to 3 hours after quinine administration. Quinetine is apparently only excreted in the first hours of quinine elimination. Quinine incubated with minced sheep liver is also partly converted to quinetine, although **quinetine** given by mouth is apparently destroyed (Lipkin, 1919).

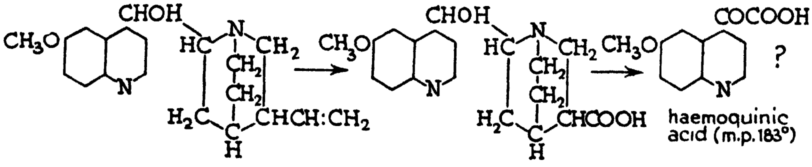
It appears therefore that quinine after oxidation to quinetine is split *in vivo* to its quinoline and quinuclidine moieties. The quinoline fragment is probably partly excreted as haemoquinic acid, but the fate of the quinuclidine fragment is unknown.

Quinine may also undergo another type of oxidation for Kelsey, Geiling, Oldham and Dearborn (1944) have found that when it is incubated with rabbit liver it is converted into a substance having a m.p. 247·5–248·5° and $[\alpha]_D - 65{\cdot}5^\circ$ (in ethanol). This substance is probably a hydroxyquinine, in which the quinoline nucleus has been oxidised to a phenol. Mead and Koepfli (1944) suggest that this compound is 2′-hydroxy-6′-methoxy-3-vinylruban-9-ol.



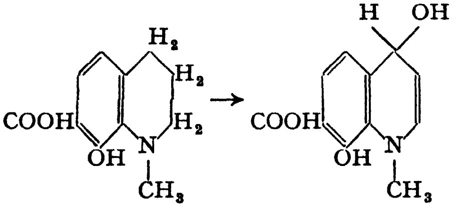
hydroxyquinine

On the known facts the degradation of quinine may be represented as follows :—

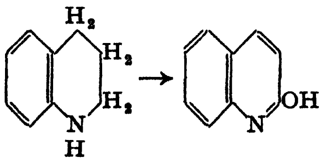


quinine quinetine (m.p 281·2°; $[\alpha]_D$ -122·6)

**Hydrogenated Quinolines.** The fate of a hydrogenated quinoline derivative was studied as early as 1888 by Krokilowsky and Nencki (1888). These workers fed **1-methyl-8-hydroxy-1:2:3:4-tetrahydroquinoline-7-carboxylic acid** to men and dogs and they found that, whilst 70–80% was excreted unchanged, some was partly dehydrogenated and oxidised to 1-methyl-4:8-dihydroxy-1:4-dihydroquinoline-7-carboxylic acid which was isolated from the urine.

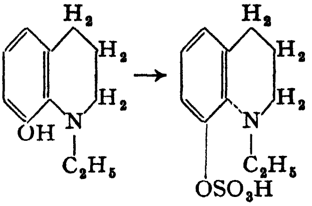


The work of Krokilowsky and Nencki suggested that the hydrogenated pyridine ring of quinoline could be dehydrogenated to an aromatic ring. The truth of this suggestion has been proved by Bernhard (1939), who found that by the dog 25% of injected **1:2:3:4-tetrahydroquinoline** is excreted as 2-hydroxyquinoline.



On the other hand, **1:2:3:4-tetrahydro*iso*quinoline** is completely demolished *in vivo*, for none of it or its possible metabolites can be detected in the urine. The fully hydrogenated compounds, **decahydro-quinoline**, **-*iso*-quinoline** and **-quinaldine** are also completely oxidised in the dog, and no trace of dehydrogenated compounds can be detected.

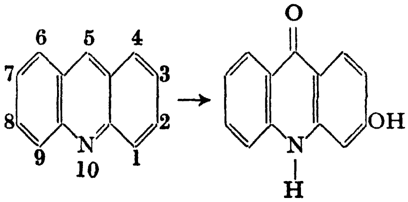
**Karine,** the hydrochloride of **N-ethyl-8-hydroxy-1:2:3:4-tetrahydroquinoline** is said to be excreted both as an ethereal sulphate and a glucuronide (Mering, 1885). The ethereal sulphate has been isolated as a potassium salt.



## ACRIDINE AND ITS DERIVATIVES

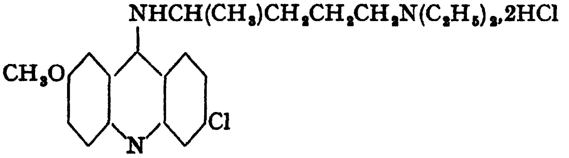
Acridine and its derivatives, e.g. **acriflavine** (trypaflavin or 10-methyl-2:8-diaminoacridinium chloride) and **rivanol** (3-ethoxy-5:8-diamino-acridine), are said to be excreted as ethereal sulphates and glucuronides (Shigenobu, 1932), indicating oxidation to phenolic substances.

According to Führer (1904) **acridine** is oxidised in the animal body to a hydroxyacridone which is probably 5-keto-2-hydroxy-5:10-dihydroacridine.

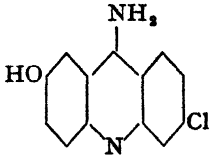


The latter compound is excreted mainly as an ethereal sulphate which accounts for about 20% of a 1 g. dose of acridine. The possible methylated product, methylacridinium hydroxide, and possible degradation products such as quinoline and pyridine carboxylic acids have not been detected.

**Mepacrine** (atabrine, atebrin). One of the most important drugs containing the acridine nucleus is the antimalarial mepacrine[1] or 2-chloro-5-diethylaminoisopentylamino-7-methoxyacridine dihydrochloride.



mepacrine



*in vivo* degradation product of mepacrine

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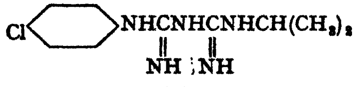
[1] The new antimalarial drug, paludrine, is excreted partly unchanged by human beings (Spinks and Tottey, 1946).



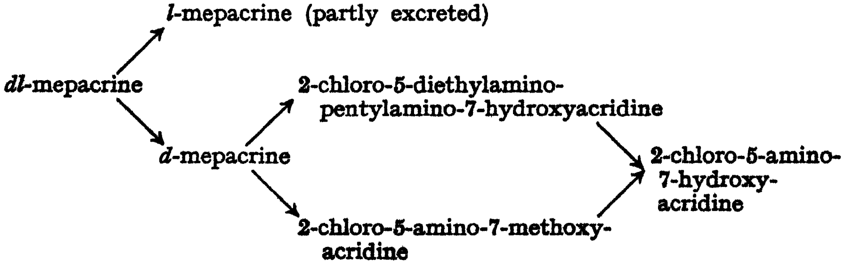
paludrine

When administered to man or animals it imparts an intense yellow colour to the urine, which is also fluorescent. The exact nature of the metabolites of mepacrine excreted in the urine is unknown.

In the dog some 5% of the drug is excreted (Scudi and Jelinek, 1944), and of this a quarter is unchanged mepacrine. From a study of the absorption spectra of the substances extracted by *iso*amyl alcohol from dog urine rendered alkaline by sodium hydroxide, Scudi and Jelinek were able to distinguish four metabolites, which they designate A, $B_1$, $B_2$ and C. A was unchanged mepacrine ; $B_1$ was probably phenolic, possibly formed by demethylation of the 7-methoxy group, the 5-diethylamino*iso*pentylamino group being intact ; in $B_2$ the basic side-chain was probably partly degraded ; $B_1$ and $B_2$ were minor metabolites and constituted some 2–5% of the acridines eliminated. C, the main metabolite in the dog, appears to be phenolic in character and forms a picrate. In the rat, about 2% of orally administered mepacrine (25 mg./kg.) is excreted and 90% of this is unchanged. The rabbit excretes in 5 hours about 1% of administered mepacrine, and five times as much of fraction C is eliminated as of mepacrine ; fractions $B_1$ and $B_2$ are not present in rabbit urine. Fraction C from the rabbit is not identical with fraction C from the dog. In the human, equal amounts of fraction C and unchanged mepacrine are excreted. According to Hammick and Firth (1944) certain specimens, but not all, of urine from malaria patients treated with mepacrine contain 2-chloro-5-amino-7-hydroxyacridine, which may be identical with fraction C of Scudi and Jelinek. The occurrence of this substance means that the 7-methoxyl group of mepacrine has been demethylated and the diethylamino*iso*pentyl group has been removed. Mepacrine itself is a *dl*-mixture, but the unchanged mepacrine excreted in human urine consists entirely of the *l*-isomer ($[\alpha]_D$ — 368° for the hydrochloride) (Hammick and Chambers, 1945). Thus it appears probable that the *d*-isomer undergoes more extensive degradation *in vivo* than its enantiomorph.

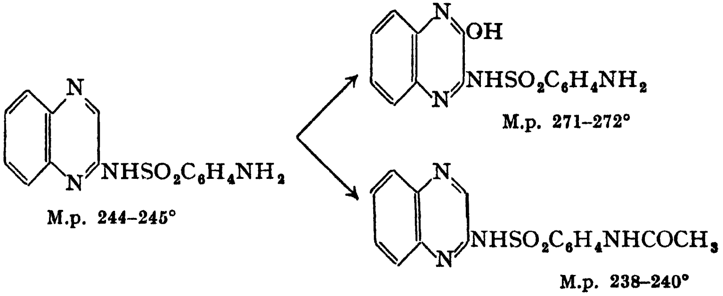
As a result of polarographic examination of the fractions separated by chromatography from human mepacrine urine, Hammick and Mason (1945) have obtained strong evidence to show that fraction $B_1$ of Scudi and Jelinek is 2-chloro-5-diethylamino*iso*pentylamino-7-hydroxyacridine, fraction $B_2$ is 2-chloro-5-amino-7-methoxyacridine and fraction C is 2-chloro-5-amino-7-hydroxyacridine.

Without reference to the quantitative aspects of the problem, the degradation of mepacrine on the evidence available can be represented as follows :—


*l*-mepacrine (partly excreted)
*dl*-mepacrine
2-chloro-5-diethylamino-pentylamino-7-hydroxyacridine
*d*-mepacrine
2-chloro-5-amino-7-hydroxy-acridine
2-chloro-5-amino-7-methoxy-acridine

## QUINOXALINE DERIVATIVES

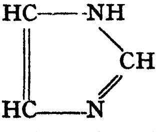
**Sulphaquinoxaline** (2-sulphanilamidoquinoxaline) appears to be the only compound containing the quinoxaline nucleus whose fate in the body has been studied. Like quinoline, it appears to be oxidised in the dog to hydroxysulphaquinoxaline in which the hydroxyl group is present in the heterocyclic ring (Scudi and Silber, 1944). In addition to 2-sulphanilamido-3-hydroxyquinoxaline, the dog excretes unchanged sulphaquinoxaline, N⁴-acetylsulphaquinoxaline (m.p. 238–240°) and unidentified conjugates of the hydroxy compound.



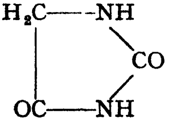
## COMPOUNDS CONTAINING THE IMIDAZOLE OR GLYOXALINE RING

Compounds containing this five-membered ring are not uncommon in nature and perhaps the most familiar examples of such compounds are the purines and the amino acid histidine.

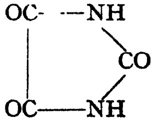
The fate of **imidazole** itself has been investigated by Novello, Harrow and Sherwin (1926) who were interested only in whether or not the compound was methylated *in vivo*. They found that it was not methylated in the dog or the rabbit. The metabolism of glyoxaline formaldehyde has already been mentioned in the section on furfural (see p. 196).



imidazole or glyoxaline

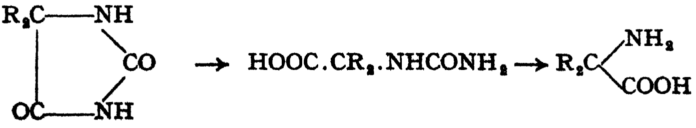


hydantoin



parabanic acid

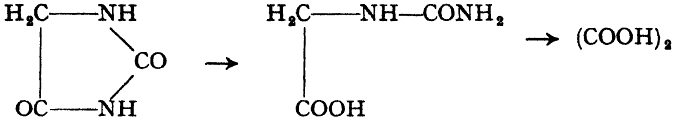
**Hydantoin Derivatives.** Compounds containing the hydantoin structure in many cases suffer, in the animal body, the opening of the imidazole ring with the formation of hydantoic acids which may be further degraded by the loss of $—CONH_2$ to amino acids. This general reaction may be represented as follows :—

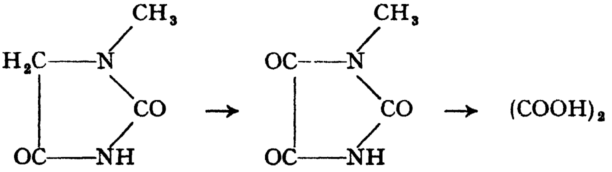


$$\longrightarrow HOOC.CR_2.NHCONH_2 \longrightarrow R_2C\begin{matrix}NH_2\\COOH\end{matrix}$$

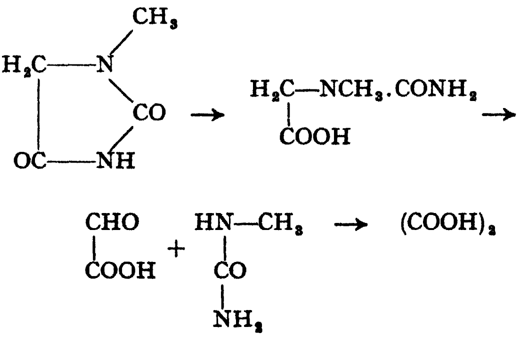
**where R = H or a substituent.**

**Hydantoin** itself is excreted by dogs to the extent of about 50% as hydantoic acid (Gaebler and Keltch, 1926), but if hydantoin is substituted, as in the case of β-**methylhydantoin,** only small amounts of the substituted compound are excreted unchanged. Apparently oxidation fails in the absence of the methyl group. According to Gaebler and Keltch, methylhydantoin is probably oxidised to methylparabanic acid, some of the latter forming oxalic acid, the output of which is increased. These workers are also of the opinion that methylhydantoic acid is not an intermediate in the oxidation of methylhydantoin, and the former compound is probably transformed first to methylhydantoin which is then oxidised to methylparabanic acid.


hydantoin hydantoic acid

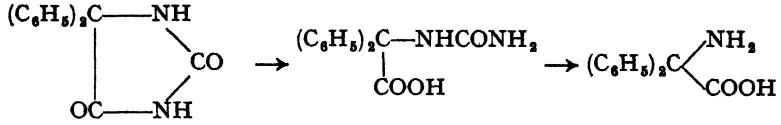

methylhydantoin methylparabanic acid

The alternative route for the degradation of methylhydantoin, i.e. *via* methylhydantoic acid, methylurea and glycollic acid to oxalic acid has not, however, been disproved.



**5:5-Diphenylhydantoin** or "dilantin" is a drug which is used in the treatment of epilepsy. In man and the dog, after oral administration or intravenous injection, only 30–35% can be accounted for by products of metabolism present in the urine (Hine and Kozelka, 1943). About

1–4% is excreted unchanged, 1–5% as diphenylhydantoic acid and 10–25% as α-aminodiphenylacetic acid.



Other derivatives of diphenylhydantoin behave similarly, forming the corresponding hydantoic acids and finally being degraded to amino acids (see Table 27).

TABLE 27

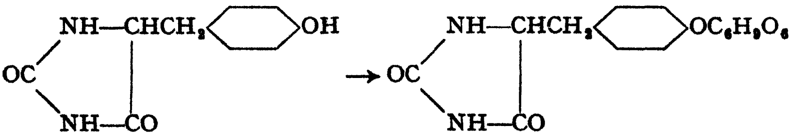
*The Excretion of Derivatives of* 5:5-*Diphenylhydantoin*
(*Hine and Kozelka,* 1943)

| Compound | Percentage excreted | | |
|---|---|---|---|
| | unchanged | as hydantoic acid | as amino acid |
| Dilantin | 3·2 | 3·9 | 22·9 |
| 1 Acetyl-5:5-diphenylhydantoin | 4·1 | 5·8 | 20·1 |
| 2-Thio- ,, ,, | 4·5 | 6·6 | 25·1 |
| 2-Dihydro- ,, ,, | 0 | 0 | 25·7 |
| 3-methyl- ,, ,, | 3·3[1] | 2·0 | 13·3 |
| 5-5-di-*p*-anisylhydantoin | 2·2 | 1·2 | 4·4 |
| 5:-ethyl-5-phenylhydantoin | 15·0 | 1·1 | 30·1 |

[1] Demethylated.

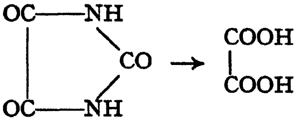
**3-Methyl-5:5-diphenylhydantoin** is demethylated before it undergoes further degradation and none of the methylated compound is excreted unchanged.

According to Ichihara and Tamura (1933), **tyrosinehydantoin** forms a conjugated glucuronide in the rabbit. In addition to this it increases the output of ethereal sulphates. It is probable that in this case it is the phenolic hydroxyl of the tyrosine moiety which undergoes conjugation. The glucuronide is laevorotatory, its potassium salt, $KC_{16}H_{17}O_9N_2$, showing $[\alpha]_D - 98°$. It is hydrolysed by acid to tyrosinehydantoin and glucuronic acid. It is probably 5-(*p*-glucuronosidobenzyl)-hydantoin.



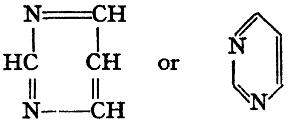
tyrosinehydantoinglucuronide

**Parabanic acid** or trioxyimidazole when fed to animals is excreted to a great extent unchanged (Lewis, 1915; Cerecedo, 1931). Part of it, however, is broken down in dogs to oxalic acid, but it causes no alteration in the urea output. There is a rise in the ammonia excretion which can be accounted for by the increased output of oxalic acid (Cerecedo).

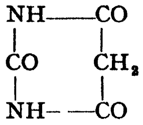


## PYRIMIDINE DERIVATIVES

From the biological point of view the pyrimidine ring is a structure of fundamental importance, for it occurs as a component of the nucleic acids and consequently of all nucleated cells, plant and animal. Furthermore, it is the basic structure of a large group of drugs, the barbiturates, which have a wide field of application in medicine.



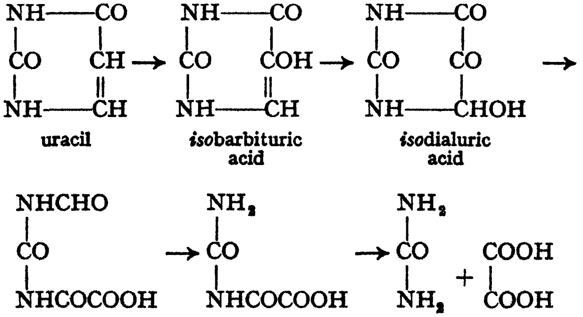
pyrimidine



barbituric acid

Little is known regarding the fate of the unoxygenated pyrimidine ring and most studies have been confined to the dioxy- and trioxypyrimidines. The latter compounds are, in general, degraded *in vivo* by opening of the ring followed by hydrolysis to urea and oxalic acid. The extent to which this degradation takes place is dependent upon other groups present, although quantitative information at present is somewhat lacking.

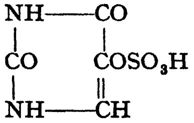
**Uracil, Isobarbituric Acid and Related Compounds.** According to Cerecedo (1930, 1931*a*) **uracil** is mainly converted into urea and oxalic acid in the dog for these two compounds appear in the urine in enhanced quantities after its oral administration. The suggested scheme for its metabolism in the dog is as follows :—



uracil *iso*barbituric acid *iso*dialuric acid

formyloxaluric acid oxaluric acid

The intermediate compounds in the above scheme, namely ***iso*****barbituric**, ***iso*****dialuric** and **formyloxaluric** acids, have also been fed to dogs (Cerecedo, 1930; Stekol and Cerecedo, 1931). All three cause a rise in the urea output, urea being the main product of their metabolism. They have an interesting effect on the various sulphur fractions of the urine. *Iso*barbituric acid behaves like a phenol,[1] causing an increased output of ethereal sulphate at the expense of the inorganic sulphate fraction both in the dog and in man (Stekol and Cerecedo, 1933). The sulphate was isolated in the crude state from dog urine by Cerecedo (1930) and has probably the following structure:—



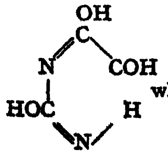
*iso*barbituric acid
ethereal sulphate

*Iso*dialuric and formyloxaluric acids do not form ethereal sulphates. All these acids cause a drop in the neutral sulphur output in the dog, although the drop in neutral sulphur after *iso*barbituric acid administration is not evident in man. No explanation of this effect on the neutral sulphur output of dogs is as yet forthcoming, but Stekol and Cerecedo (1931) assume that during the metabolism of the above three compounds a sulphur-containing compound is involved which is ordinarily excreted in the neutral sulphur fraction of the urine. This drop in the neutral sulphur output in dogs after ingesting *iso*barbituric acid was not confirmed by Lawrie and Pirie (1932) although they were able to confirm the increased output of ethereal sulphate. These differences may be explicable by the different types of diet on which the animals were kept during the experiments by the two groups of workers (Stekol and Cerecedo, 1933). Formyloxaluric acid is toxic to dogs, but this does not necessarily preclude it from being an intermediate in the metabolism of uracil.

**Thiouracil and related compounds.** It has recently been shown that certain compounds which contain the $-\overset{|}{N}-\overset{|}{C}=S$ grouping influence the activity of the thyroid gland and potassium thiocyanate, thiourea,[2] thiouracil and 4-methylthiouracil have been used in the treatment of hyperthyroidism (Astwood, 1943; Mackenzie and Mackenzie, 1943; Foulger and Rose, 1943; Leys, 1945). Thiouracil and its 4-methyl derivative are the least toxic of these compounds and have been more

---

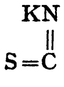
[1] The formula of *iso*barbituric acid can also be written as



which is that of a trihydric heterocyclic phenol.

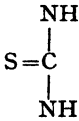
[2] Tetramethylthiourea, but not diethylthiourea, has been used successfully in the treatment of thyrotoxicosis (Williams, 1945).

widely used than the others, but it appears from experiments on rats that thiobarbital (5:5-diethyl-2-thiobarbituric acid) may be less toxic and slightly more effective in its antithyroid properties than thiouracil (Astwood, Bissel and Hughes, 1945). It is suggested that these compounds lower the basal metabolic rate by interfering with the synthesis of thyroxine (Himsworth, 1943; Campbell, Landgrebe and Morgan, 1944).



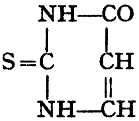
$$\begin{matrix}KN\\ \| \\ S{=}C\end{matrix}$$

potassium thiocyanate

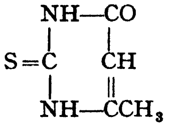


$$\begin{matrix}NH_2\\ | \\ S{=}C\\ | \\ NH_2\end{matrix}$$

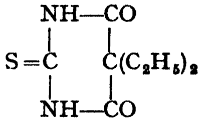
thiourea



thiouracil



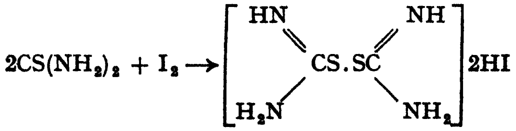
4-methylthiouracil



thiobarbital

The possibility exists that all these compounds may be converted into a common metabolite which is the effective therapeutic agent, but on our present knowledge of their fate in the body no conclusion regarding this possibility can be made.

**Thiourea** undergoes practically no alteration *in vivo*. It is quickly absorbed from the intestine, diffusing widely in the tissues and is then rapidly excreted unchanged. In man, a 1 g. dose is almost completely eliminated in the urine in 24 hours, 75% being excreted in 10 hours and 96% in 24 hours (Campbell *et al.*, 1944). Concerning its mode of action, Campbell *et al.* have suggested that, since thiourea has a peculiar chemical affinity for iodine, it combines *in vivo* with iodine which would normally be used to form diiodotyrosine. The product of such a reaction would be formamidine disulphide hydriodide thus :—



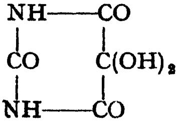
$$2CS(NH_2)_2 + I_2 \rightarrow \left[\begin{matrix}HN & & & & NH\\ & \diagdown & & \diagup & \\ & & CS.SC & & \\ & \diagup & & \diagdown & \\ H_2N & & & & NH_2\end{matrix}\right]2HI$$

There is, however, no evidence to show that this reaction takes place in the body (Williams, Weinglass and Kay, 1944). An observation which may have some connection with the above suggestion is that when thiouracil and potassium iodide are administered simultaneously to guinea-pigs, there is a tendency for increased storage of thiouracil in the thyroid tissue; simultaneous administration of thyrotrophic hormone, on the other hand, tends to decrease storage of thiouracil (Williams *et al.*, 1944).

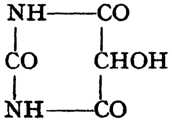
It is probable that **thiouracil** undergoes considerable change in the body, but the exact nature of its metabolites is unknown. On analogy with uracil, it should give rise to thiourea and oxalic acid. According to Williams and Kay (1944), one-half of an oral dose of 200 mg. is destroyed in man and about one-third is excreted unchanged. There is also an increase in urinary neutral sulphur, which is partly due to unchanged thiouracil, and a decrease in organic and inorganic sulphates.

With daily doses of 1·0–1·2 g. of thiouracil, some 60–70% is excreted by man as material giving a bluish-green colour with Grote's reagent (Anderson, 1944). This reagent does not distinguish between thiourea and thiouracil because it gives a colour with all compounds containing the C=S group (Grote, 1931). Thiouracil urine, however, does not contain thiourea (Anderson, 1944), for it does not give the colour test of Campbell *et al.* (1944), which is based on the conversion of thiourea by nitrous acid into thiocyanate, which is detected by ferric chloride.

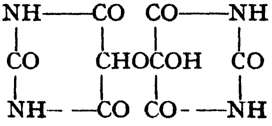
**Alloxan** is a compound which has come to the forefront in recent years owing to its ability to produce an experimental diabetes in animals (Dunn, McLetchie and Sheehan, 1943 ; Dunn and McLetchie, 1943). It apparently has a destructive action on the islets of Langerhans of the pancreas and produces a diabetic state which can be controlled by insulin. A single intravenous dose of at least 200 mg./kg. alloxanal ways causes serious damage to the islets of Langerhans in rabbits. The $\beta$-cells are affected and may become necrosed. The $\alpha$-cells are generally less affected. A temporary hyperglycaemia almost always occurs in the first two hours after doses sufficient to produce any damage of the islets (Dunn, Duffy, Gilmour and Kirkpatrick, 1944). In the dog alloxan is partly excreted as urea but does not increase the ethereal sulphate output of the urine. There is, however, a decrease in the inorganic sulphate output of the urine without a compensatory increase in the ethereal sulphate (Cerecedo, 1931). Cerecedo therefore assumes that alloxan is probably excreted in the bile, perhaps as an ethereal sulphate. There is evidence to show that it may be partly converted to alloxantin and eliminated in the urine as murexide.



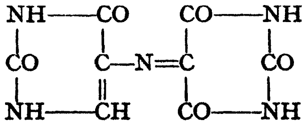
alloxan



dialuric acid



alloxantin



murexide

The results of Brückman and Wertheimer (1945) suggest that alloxan may be partly converted in the rat to dialuric acid, and *vice versa.* In the rat, diabetes is caused by a number of compounds closely related to alloxan ; these are methylalloxan, alloxantin, dimethylalloxantin, dialuric acid and methyldialuric acid. Other closely related compounds such as dimethylalloxan, dimethyldialuric acid, violuric acid and murexide are not diabetogenic. The minimum diabetogenic dose (50-70 mg./kg.) is about the same for all the active compounds including alloxan. This suggests, that dialuric acid, alloxantin and alloxan may be interconvertible *in vivo* or, that all the active compounds have some property (or metabolite) in common which enables them to affect the islet cells.

**Alloxantin** was fed to man in an experiment carried out nearly one hundred years ago by Wöhler and Frerichs (1848), who found that it was broken down to urea. According to Lusini (1894, 1895) it is partly destroyed *in vivo* and partly excreted as murexide. In dogs (Cerecedo, 1931) it causes a decrease in the inorganic sulphate fraction of the urine as in the case of alloxan and is possibly excreted in the bile as an ethereal sulphate. It also causes a decrease in the output of urea and total nitrogen, but this may be due to its exerting a slight toxic action on the kidney (see above for effect on the pancreas). Free alloxantin is present in the urine and the dogs void a red-coloured urine. It appears probable that dogs excrete it partly as murexide.

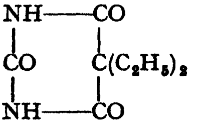
## THE SUBSTITUTED BARBITURIC ACIDS

The studies on the metabolism of the barbituric acid drugs have indicated that there is probably a close relationship between the depth and length of anaesthesia that different barbiturates produce and their fate in the animal body. In many cases, however, intermediate compounds in their metabolism are as yet unknown.

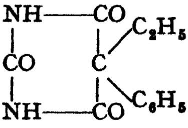
**Veronal** (barbital, 5:5-diethylbarbituric acid) is excreted unchanged in the urine to the extent of 70–90%, but no intermediate decomposition product has yet been detected (Weese, 1933).

**Luminal** (5-phenyl-5-ethylbarbituric acid), which produces lengthy sleep and is an anti-epileptic, is largely decomposed in the body and only 11–25% is excreted unchanged in the urine.

No intermediate metabolite is known.



veronal or barbital

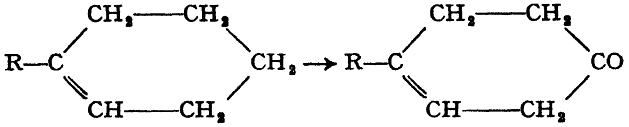


luminal

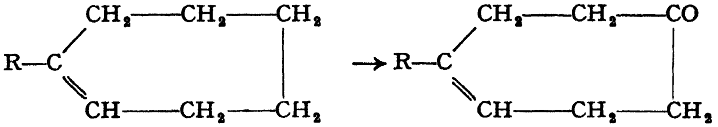
**Phanodorm** (5-*cyclo*hexenyl-5-ethylbarbituric acid) is excreted to the extent of 2·5–6·3% unchanged and 12–19% as a metabolite which is probably 5-*cyclo*hexenonyl-5-ethylbarbituric acid. 80% of the drug is unaccounted for (cf. Weese).

**Medomin** (5-*cyclo*heptenyl-5-ethylbarbituric acid) is also oxidised in the body forming 5-*cyclo*heptenonyl-5-ethylbarbituric acid. About 1% of medomin is excreted as the heptenonyl derivative. *Cyclo*heptenonylethylbarbituric acid has no hypnotic effects and is twenty times less toxic to mice than medomin (Pulver, 1942).

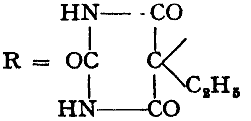
The metabolic fate of phanodorm and medomin gives us some information as to the mode of oxidation of the *cyclo*hexene and *cyclo*heptene rings. The first stage in the oxidation of these structures is obviously oxidation of a ring $>CH_2$ group to $>CO$. It is not clear, however, in these cases, which $CH_2$ group of the *cyclo*hexene and *cyclo*heptene rings is oxidised ; there are four possibilities in phanodorm and five in medomin :—



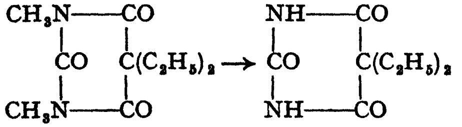
phanodorm



medomin

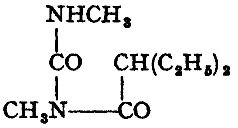


**N-Methyl Substituted Barbitals.** The anaesthetic effects of **N-methyl-** and **N:N′-dimethyl-barbital** are qualitatively similar in that the apparent effects of small doses disappear quickly, whereas the first intense effects of large doses are followed by prolonged effects of lesser intensity. There are several possible explanations of these results and the two most plausible are :—(1) the rate of inactivation, while initially high, may decrease as the duration of anaesthesia increases ; (2) these substances may be transformed *in vivo* not to inactive but to less active compounds, the latter being responsible for the after-effects following large doses, that is the course of anaesthesis is related to their metabolism. The second of these explanations has been tested by Butler and Bush (1939). The possible change that the above compounds may undergo *in vivo* is demethylation with the production of barbital, as Butler and Bush suggest, or opening of the pyrimidine ring as suggested by Shonle, Keltch, Kempf and Swanson (1933). Butler and Bush therefore tested the anaesthetic activity in mice of the open-chain compounds N-diethylacetyl-N:N′-dimethylurea and N-phenylethylcarboxyacetyl-N′-methylurea and found that they had little anaesthetic activity and could not therefore be responsible for the after-effects mentioned above. However, the corresponding methylated compounds were active and it seemed likely that the after-effects were due to these less active compounds. Butler and Bush therefore injected anaesthetic doses of N-methylphenobarbital, N-methylbarbital and N:N′-dimethylbarbital into dogs. With the first compound they found that the urine contained none of the original compound, but they were able to isolate pure phenobarbital in quantities equivalent to 4% of the dose of original drug. In the case of N-methylbarbital, as much as 44% of it was isolated from the urine as the demethylated compound, barbital (veronal), and about 2–3% of the original drug was also isolated. In the case of N-N'dimethylbarbital, 3% of it was isolated as barbital.

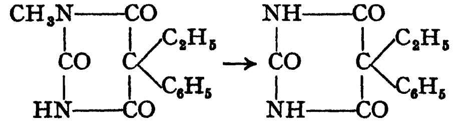


N : N'-dimethylbarbital

barbital (veronal)

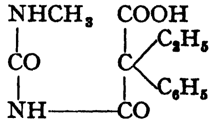


N-diethylacetyl-N:N'-dimethylurea



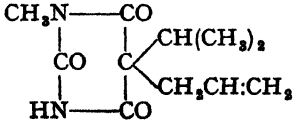
N-methylphenobarbital

phenobarbital

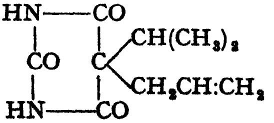


N-phenylethylcarboxyacetyl-N'-methylurea

Dealkylation also occurs *in vivo* with N-ethyl derivatives, but occurs only to a small extent with N-*n*-propyl-, N-*iso*-propyl-, N-allyl-, N-*n*-butyl- and N-phenylbarbital (Bush and Butler, 1940). It appears, therefore, that the short-duration anaesthetic action of certain barbiturates can be largely attributed to the *in vivo* removal of the methyl group from the nitrogen atom with the formation of the less active 5.5-disubstituted barbituric acid. However, in the case of **narconumal** (N-methyl-5-allyl-5-*iso*propylbarbituric acid) this mechanism is unlikely to explain its short-duration narcotic action, and it appears that this drug is mainly inactivated by reactions other than demethylation (Butler and Bush, 1940*a*). Demethylated narconumal is 5-allyl-5-*iso*propylbarbituric acid (alurate), a drug of high activity and long duration of action. If demethylation were the principal reaction responsible for the inactivation of narconumal, the residual narcotic effects due to alurate would be conspicuous. In the mouse the narcotic effect of narconumal after being high initially soon becomes much less intense than that of an equimolecular quantity of alurate, thus indicating that alurate is unlikely to be a metabolite of narconumal. In the dog, up to 2% of intravenously injected narconumal is excreted in the urine as alurate, and since 7% of injected alurate can be recovered from the urine of dogs, then less than 30% of injected narconumal is likely to be demethylated to alurate (Butler and Bush, 1940*a*).



narconumal



alurate

## PURINE DERIVATIVES

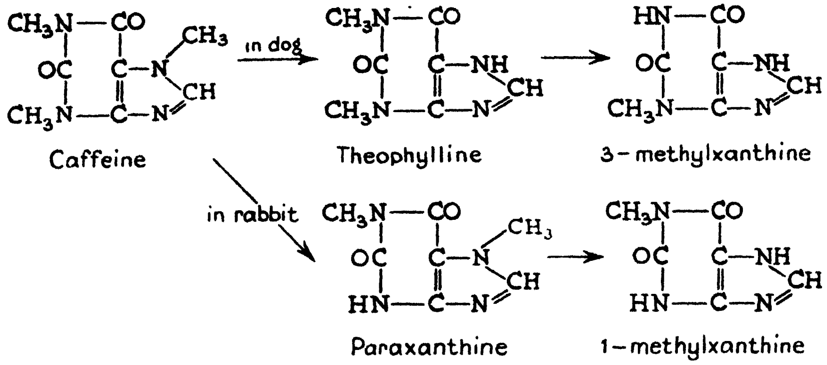
The metabolism of those purine derivatives which occur in the nucleic acid molecule and their breakdown to uric acid in the primates and to allantoin in the lower animals is now well known. Here it is proposed to give an account of those purine derivatives which are normally foreign to the body. Methylated xanthines occur in foodstuffs, particularly in beverages such as tea, coffee and cocoa, and in the ordinary course of life man absorbs into his system appreciable amounts of **caffeine**, **theophylline** and **theobromine** (1:3:7-trimethyl-, 1:3-dimethyl- and 3:7-dimethylxanthine). Besides occurring in food, the methylated xanthines are widely used as drugs. It has been suggested that the pharmacological properties of the methylated xanthines can be correlated with the positions of the methyl groups in the xanthine molecule. Thus caffeine appears to possess three effects, (1) it stimulates the central nervous system, (2) it is a diuretic and (3) it accelerates the heart by a direct stimulation of cardiac muscle. Apparently the first effect is associated with the 1-methyl group and is not shown by theobromine (3:7-dimethyl-) ; the second effect with the 3-methyl group ; and the third with the 7-methyl group and is not shown by theophylline (1:3-dimethyl-). All three compounds as well as 3-methylxanthine are diuretics.

In man, the 3-methyl is more readily removed than the 1- and 7-methyl groups, for, although the 1:3:7-, 1:3- and 3:7-methyl derivatives are ingested in food, the urine contains the 1:7, 1- and 7-methyl derivatives, compounds which could not have been derived from either exogenous or endogenous nucleic acids.

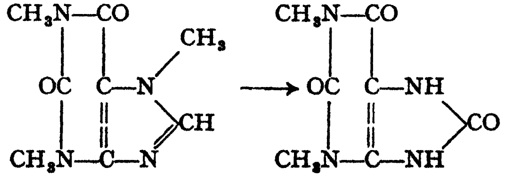
The fate of the methylated xanthines in the dog is apparently different from that in the rabbit, for whilst a methyl group in the 3-position of the xanthine molecule is easily removed in the rabbit, it appears to possess considerable stability in the dog. On the other hand, methyl groups in the 1- and 7-positions tend to resist demethylation in the rabbit, although in the dog they are less resistant than the 3-methyl group. Krüger (1899) found that when caffeine was fed to dogs the main metabolites found in the urine were theophylline (1:3-dimethylxanthine) and 3-methylxanthine ; small amounts of theobromine (3:7-dimethylxanthine) and paraxanthine (1:7-dimethylxanthine) were also eliminated. In the rabbit, however, the metabolites were 1:7-dimethylxanthine, heteroxanthine (7-methylxanthine) and 1-methylxanthine (Krüger, 1899*a*) ; no 3-methyl derivatives were found.

These differences are further illustrated when 3:7-dimethyl-, 1:7-dimethyl- and 3-methylxanthines are fed (Krüger and Schmidt, 1899). In the rabbit, theobromine is excreted mainly as 7-methylxanthine accompanied by only small amounts of 3-methylxanthine, whereas in the dog the position is reversed. Man is similar to the rabbit in this respect, for on ingesting theobromine twice as much 7-methyl- as 3-methylxanthine is excreted (Krüger and Schmidt, 1901).

The 1-position is more resistant to demethylation than the 7 in the rabbit, for the main metabolite of 1:7-dimethylxanthine in this animal is 1-methylxanthine ; none of the 7-derivative is found. The rabbit, however, does excrete some administered 3-methylxanthine unchanged, for Krüger and Schmidt (1899) found that a number of rabbits which had collectively received 20·6 g. of 3-methylxanthine excreted only 4·6 g. unchanged, although no other metabolite was detected.



It appears probable that caffeine and theophylline, but not theobromine, are also oxidised in man to methylated uric acids. According to Buchanan, Christman and Block (1945) ingested caffeine and theophylline (but not theobromine) cause an increased excretion of phosphotungstic acid-reducing material, but not, as shown by means of the specific enzyme uricase, of true uric acid. These workers suggest that caffeine and theophylline undergo, in man, both oxidation in position 8 and demethylation with the formation of 1-methyl-, 3-methyl- and 1:3-dimethyluric acids. This suggestion has been partly confirmed by Myers and Hanzal (1946) as a result of studies using the white rat and the Dalmatian dog as experimental animals. These workers found that theophylline was eliminated by the Dalmatian dog mainly as 1:3-dimethyluric acid together with a small amount of 1-methyluric acid. They found caffeine to be fairly completely demethylated at position 7 and oxidised at position 8 giving 1:3-dimethyluric acid :—

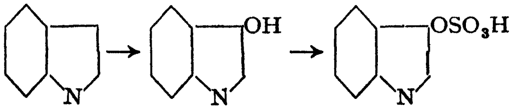


**Methylated Uric Acids.** Methylated uric acids are also demethylated in the animal body. A methyl group is more readily removed from position 3 than from any other position in the uric acid molecule. Myers and Hanzal (1946) obtained the following results, using the white rat, an animal with a high uricolytic index, and the Dalmatian dog, an animal with a low uricolytic index, as experimental animals :—

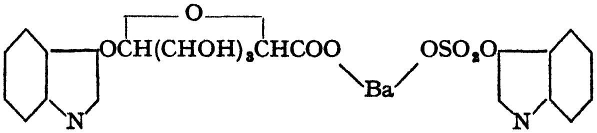
| *methylated uric acid* | *result of metabolism* | |
|---|---|---|
| 1-methyl | Excreted unchanged | |
| 3-methyl | Completely demethylated to uric acid | |
| 1:3-dimethyl | Largely unchanged; some demethylation at position 3 | |
| 3:7-dimethyl | No demethylation at position 7 | no data on positions 1 and 3 |
| 1:7-dimethyl | No demethylation at position 7 | |
| 3:9-dimethyl | Some demethylation at positions 3 and 9 | |
| 1:3:7-trimethyl | Partly demethylated at position 7. | |

## INDOLE AND ITS DERIVATIVES

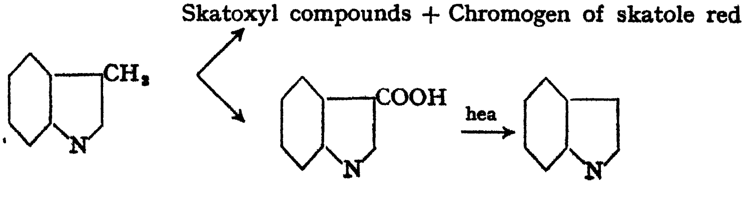
Both **indole** and **skatole** are oxidised in the animal body to phenolic compounds, which are excreted as ethereal sulphates and glucuronides. The product of oxidation of indole is indoxyl or β-hydroxyindole, but that of skatole is unknown, although it is usually referred to as skatoxyl (Baumann, 1877; Mester, 1888).



Indoxyl occurs normally in urine as an ethereal sulphate and the well-known urinary indican is potassium indoxyl sulphate (G. Hoppe-Seyler, 1916). The glucuronide of indoxyl is also known and has been isolated as the double barium salt (formula below) of indoxylglucuronide and indoxyl sulphuric acid (Neuberg and Schwenk, 1917) from the urine of dogs receiving indole. The salt is laevorotatory and its rotation changes with time ($[\alpha]_D - 54{\cdot}9° \xrightarrow{24\text{ hrs.}} - 34°$).

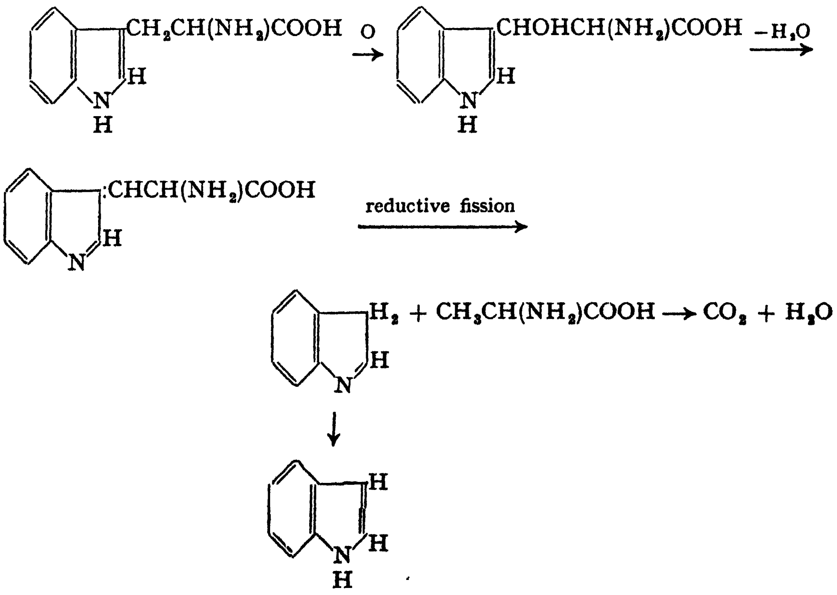


The urines of animals which have received skatole (β-methylindole) develop a red colour on the addition of hydrochloric acid. The constitution of this pigment, skatole red, is unknown. Distillation of skatole urine results in the formation of indole and it appears probable that this indole is derived from the decomposition of indole-β-carboxylic acid (see p. 224) formed by the oxidation of the β-methyl group of skatole.



The indole and skatole excreted by the animal organism is said to be formed in the alimentary tract by the bacterial decomposition of tryptophan. The indole and skatole thus formed being absorbed and oxidised to indoxyl and skatoxyl. The conversion of tryptophan to indole in the alimentary tract is generally reckoned to take the following path :—tryptophan ⟶ indole-β-propionic acid ⟶ indole-β-carboxylic acid ⟶ indole (cf. for example, Dakin, 1922*c*).

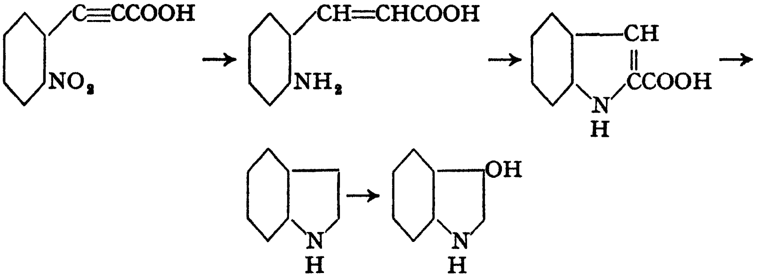
The work of Ward (1923) suggests that indole-β-carboxylic acid is not a degradation product of indole-β-propionic acid. The latter substance probably forms an α-hydroxyindole, whereas indole-β-carboxylic acid is excreted mainly unchanged, although conjugated. The work of Baker and Happold (1940) on the enzymic conversion of *l*-tryptophan to indole by *E. coli* has thrown new light on the mechanism of the transformation of tryptophan to indole. It is suggested that for the enzymic breakdown of *l*-tryptophan to indole by the tryptophanase system of *E. coli* the structural features necessary are (*a*) a free carboxyl group, (*b*) an unsubstituted α-amino group, and (*c*) a β-carbon atom capable of oxidative attack. These conditions obviously eliminate indole-β-carboxylic and -β-propionic acids as intermediates in the bacterial conversion of tryptophan to indole. Baker and Happold have therefore tentatively suggested the following scheme :—



It is suggested that the integrity of the α-amino and carboxylic groups might be needed to " fix " an amphoteric enzyme system to the tryptophan molecule. The unusual reaction in this scheme is reductive fission, which has no known analogy, but Baker and Happold point out that this reaction may not take place directly in one stage.

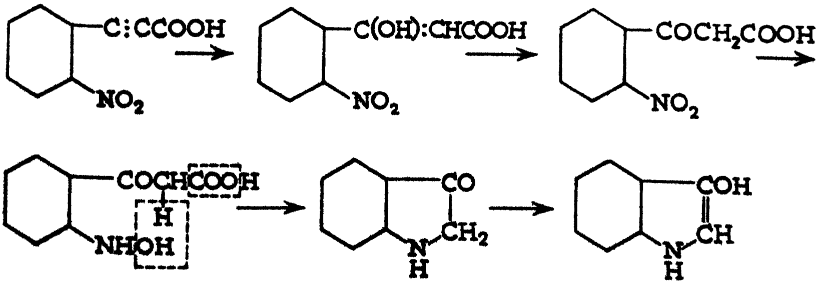
## THE CONVERSION OF *O*-NITRO DERIVATIVES INTO INDICAN *IN VIVO*

In 1882 Hoppe-Seyler made the interesting discovery that ***o*-nitrophenylpropiolic acid** was converted into indican in the animal body. Hoppe-Seyler made no suggestion as to the mechanism of this change. Later Sherwin (1923) suggested a mechanism in which the nitro group and the acetylenic link are reduced respectively to an $NH_2$ group and an ethylenic link to produce *o*-aminocinnamic acid which is then cyclised, decarboxylated and oxidised to indoxyl.



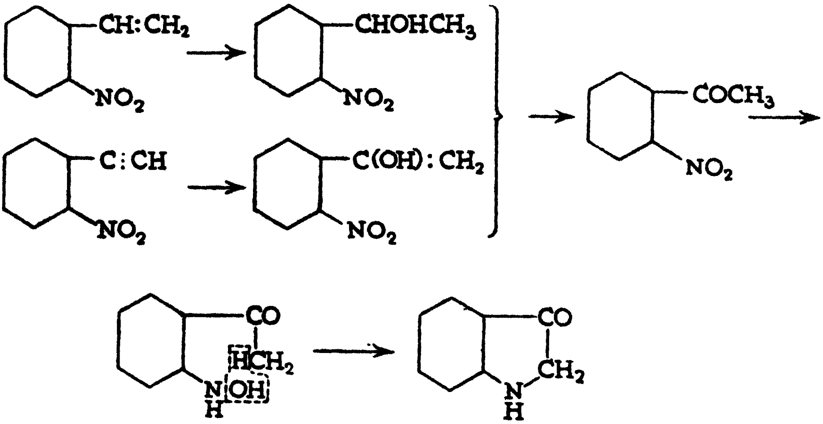
That this scheme is untenable, however, can be deduced from the fact that although the *o*-nitro compounds cause an increased excretion of indican in animals, the corresponding *o*-amino compounds do not (Böhm, 1937, 1938, 1939). It follows that the amino compound cannot be an intermediate in the formation of indican from *o*-nitro compounds. The following *o*-nitro compounds cause increased excretion of indican in the rabbit, *o*-nitrophenylpropiolic acid, *o*-nitroacetophenone, *o*-nitrophenylacetylene, *o*-nitrostyrene, *o*-nitrocinnamylformic acid and *o*-nitrobenzoylacetic acid. On the other hand, the following *o*-amino compounds do not increase the indican excretion of rabbits, *o*-aminophenylpropiolic acid, *o*-aminoacetophenone and *o*-aminocinnamic acid.

Böhm proposes that the intermediate compound which is formed before ring closure is a hydroxylamine and not an amino compound. This is not unreasonable for the biological formation of a hydroxylamine from an aromatic nitro compound has been demonstrated in the case of trinitrotoluene by Channon, Mills and Williams (1944) (p. 133). The mechanism according to Böhm for the formation of indoxyl from *o*-nitrophenylpropiolic acid is as follows:—



It will be noted that indole is not an intermediate in this scheme.

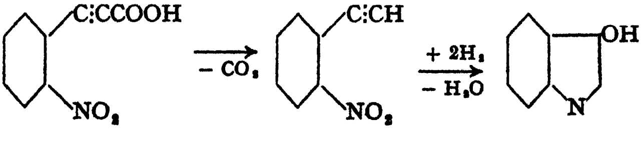
For compounds containing a two-carbon side-chain such as *o*-nitrophenyl-acetylene and -ethylene, Böhm proposes *o*-nitro and *o*-hydroxylaminoacetophenone as intermediates.



In hens *o*-nitrophenylpropionic acid does not give rise to indican; it is largely oxidised to *o*-nitrobenzoic acid and excreted as such.

Although most *o*-aminobenzene derivatives have no indoxylogenic action, Stoppani (1945) has found that *o*-aminophenylethanol is as effective an indoxylogenic agent in the dog as *o*-nitrophenylpropiolic acid or indole. Table 28 is compiled from the data of Böhm on the rabbit and of Stoppani on the dog and gives a list of *ortho* substituted nitro- and amino-benzene derivatives which are indoxylogenic. The inability of most *o*-aminophenyl derivatives to give indoxyl *in vivo* is explained by Stoppani as being due to the inability of animal tissues to produce the appropriate side-chain (containing probably a —CHO group) for coupling with the *o*-amino group.

In the abdominally eviscerated dog only *o*-nitrophenylpropiolic acid and *o*-nitrophenylacetylene produce indoxyl, and this may indicate that *o*-nitrophenylacetylene is an intermediate in the production of indoxyl from *o*-nitrophenylpropiolic acid, as proposed by Baeyer (1880), thus:—



The presence of the liver is essential for the indoxylogenic action of *o*-aminophenylethanol and of *o*-nitrobenzoylacetic acid, whereas the digestive tract is necessary for that of *o*-nitrophenylethanol, *o*-nitroacetophenone and *o*-nitrophenacetaldehyde (Stoppani, 1945).

Of indole derivatives, only indole itself and, to a slight extent, indolealdehyde form indoxyl in the dog (see Table 28).

TABLE 28

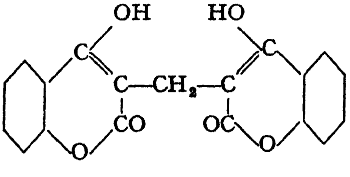
*Indoxylogenic Compounds*

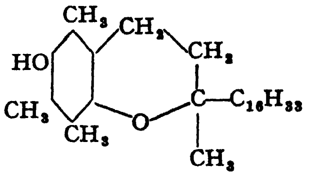
| Indoxylogenic | | Non-indoxylogenic | |
|---|---|---|---|
| Compound | Formula of side-chain | Compound | Formula of side-chain |
| *o*-Nitrophenylpropiolic acid | —C⋮CCOOH | *o*-Aminophenylpropiolic acid | —C⋮CCOOH |
| *o*-Nitrophenylacetylene | —C⋮CH | *o*-Aminophenylacetylene | —C⋮CH |
| *o*-Nitrophenylethylene | —CH:$CH_2$ | *o*-Aminophenylacetic acid | —$CH_2$COOH |
| *o*-Nitroacetophenone | —CO$CH_3$ | *o*-Nitrophenylacetic acid | —$CH_2$COOH |
| *o*-Nitrophenylethanol | —$CH_2CH_2$OH | *o*-Aminoacetophenone | —CO$CH_3$ |
| *o*-Aminophenylethanol | —$CH_2CH_2$OH | *o*-Nitrocinnamic acid | —CH:CHCOOH |
| *o*-Nitrophenylacetaldehyde | —$CH_2$CHO | *o*-Aminocinnamic acid | —CH:CHCOOH |
| *o*-Nitrocinnamoylformic acid | —CH:CHCOCOOH | *o*-Nitrobenzoylacetic acid | —CO$CH_2$COOH |
| *o*-Nitrophenylpropiolic ethyl ester | —C⋮CCOOEt | *o*-Nitrophenylpyruvic acid | —$CH_2$COCOOH |
| Indole | | Indole carboxylic acid | |
| Indole aldehyde | | Indole ethylamine | |
| | | Indole acetic acid | |
| | | Indole pyruvic acid | |
| | | Skatole | |
| | | Tryptophan | |

## COUMARIN AND RELATED DERIVATIVES

The metabolism of the coumarin nucleus is at the moment obscure. There is, however, some evidence which shows that the structure is stable *in vivo* and that the pyrone ring in some cases (in α-pyrone but not γ-pyrone compounds) does not undergo fission. At least two compounds containing the benzopyrane ring have been shown to be of physiological importance. **3:3′-Methylene-*bis*-(4-hydroxycoumarin)** or **dicoumarol** is the haemorrhagic and anticoagulant agent present in spoiled sweet-clover hay and is responsible for haemorrhagic sweet clover disease in ruminants. It has been suggested that salicylic acid is one of its possible metabolites (Link, Overman, Sullivan, Huebner and Scheel, 1943). Dicoumarol does not affect the clotting power of blood or plasma *in vitro*, and even when given orally to man or animals, its hypoprothrombinaemia-inducing action does not become detectable for 12 to 24 hours. This delayed action probably indicates its conversion *in vivo* to an active haemorrhagic agent. Chemically, the compound can be degraded to salicylic acid. Salicylic acid in single doses can induce a temporary hypoprothrombinaemia in rats on rations low in vitamin K, but this action of salicylic acid does not develop in the presence of adequate vitamin K or 2-methyl-1:4-naphthoquinone (vitamin $K_3$). It has been suggested that salicylic acid, as a metabolite of dicoumarol, is the carrier of the haemorrhagic function of the latter. The recent work of Lester (1944), however, indicates that this suggestion is probably incorrect, for he found that no salicylic acid was excreted in the urine of rats receiving dicoumarol. If it were formed salicylic acid would survive and be excreted in the urine (see p. 102).

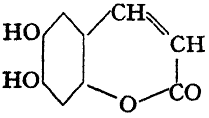
The **tocopherols** (Vitamin E) also contain the benzopyrane (chromane) structure.

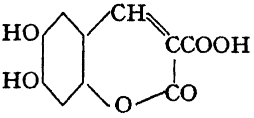

dicoumarol

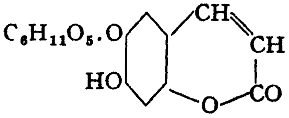

α-tocopherol

Vitamin E is readily broken down in the organism of the rat and only small amounts pass into the urine unchanged. Storage in the tissues only takes place when very high doses are given (Cuthbertson, Ridgeway and Drummond, 1940) and the fate of the rest is unknown.

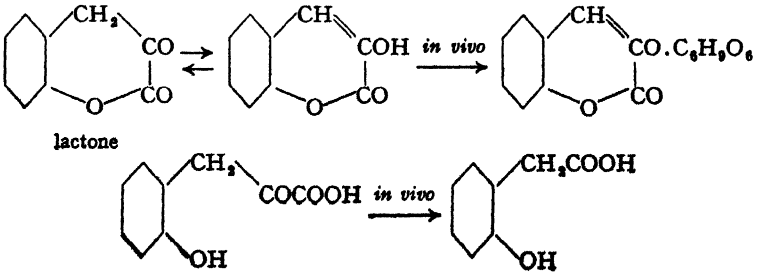
**Aesculin** is the 6-glucoside of aesculetin (6:7-dihydroxycoumarin) and occurs in the horse-chestnut and the roots of wild jasmin. In the animal body it is partly split to glucose and aesculetin, for both aesculin and aesculetin are excreted. **Aesculetic acid** (aesculetin-3-carboxylic acid), when fed, is mainly excreted unchanged (Modica, 1893).


aesculetin


aesculetic acid


aesculin

**3-Hydroxycoumarin** is converted in the human being to 3-glucuronosidocoumarin (m.p. 207° : $[\alpha]_D - 72{\cdot}05°$). There is no evidence to show that the pyrone ring is opened and hydroxycoumarin is almost quantitatively converted to the glucuronide (Flatow, 1910). 3-Hydroxycoumarin is the enol form of the lactone of *o*-hydroxyphenylpyruvic acid and its behaviour *in vivo* is in sharp contrast with that of the acid. The latter acid is oxidised almost quantitatively in the rabbit to *o*-hydroxyphenylacetic acid (see p. 117), and no trace of glucuronosidocoumarin is formed (Flatow).

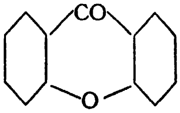


lactone

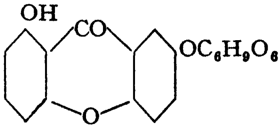
free acid

## XANTHONE DERIVATIVES

**Euxanthic Acid.** Little is known regarding the metabolism of the xanthone ring, but the occurrence in nature of the well-known compound euxanthic acid is evidence that hydroxy-xanthones, if fed to animals, are likely to be excreted in conjugation with glucuronic acid.



xanthone



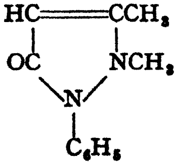
euxanthic acid

Euxanthic acid, or 4-glucuronosido-7-hydroxyxanthone, (m.p. 156–158°; $[\alpha]_D - 110°$ aqueous alcohol) is obtained from the pigment Indian yellow or " puree ", which is present in the urine of cows fed on mango leaves. Euxanthic acid occurs in the pigment mainly as the magnesium salt. Indian yellow was at one time made in large quantities in Monghir, a state of Bengal, by the simple expedient of feeding cows with mango[1] leaves. The product obtained was a source of revenue to the natives, but its production is now discouraged owing to the deleterious effects of the process on cows. Nowadays Indian yellow is consequently a rare pigment. Euxanthic acid is obviously a detoxication product in the cow of euxanthone[2] or its precursors present in mango leaves. When fed to rabbits, **euxanthone** (4:7-dihydroxyxanthone) is conjugated and excreted in the urine as euxanthic acid (Kostanecki, 1886). It has been prepared synthetically by Neuberg and Niemann (1905) from euxanthone and acetobromoglucurone.

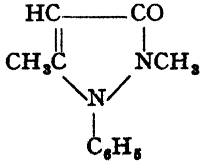
## PYRAZOLE DERIVATIVES

Derivatives of pyrazole have found great use as antipyretics, the best-known derivatives of this group being antipyrine and pyramidone.

**Antipyrine** (1-phenyl-2:3-dimethylpyrazol-5-one) is partly excreted unchanged and partly converted to hydroxyantipyrine, which is excreted combined with glucuronic acid and sulphuric acid. The position of the hydroxyl group is not known; it could only be attached to position 4 in the pyrazolone ring; alternatively it could be present in the phenyl group.



antipyrine



3-antipyrine

The glucuronide has been isolated as a laevorotatory double salt with $BaCl_2$ with the formula $(C_{17}H_{19}N_2O_8)_2,BaCl_2,H_2O$ (Lawrow, 1901).

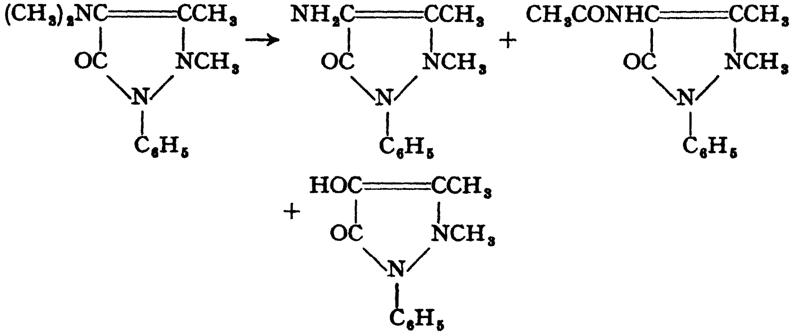
---

[1] *Mangifera indica L.*

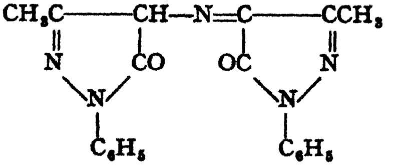
[2] Euxanthone has been isolated from the heartwood of *Platonia insignis* (Spoelstra and van Royen, 1929).

**3-Antipyrine** (1-phenyl-2:5-dimethylpyrazol-3-one) is excreted by dogs, cats and rabbits partly conjugated ; the nature of the conjugate is obscure (cf. Kobert, 1911).

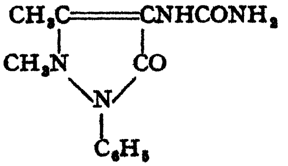
**Pyramidone** or 1-phenyl-2:3-dimethyl-4-dimethylaminopyrazol-5-one has been the subject of more recent investigations than has antipyrine. When ingested by man more than half can be accounted for by metabolites present in the urine.



The main products of pyramidone metabolism in man are 4-acetylaminoantipyrine (m.p. 198–199°) and 4-aminoantipyrine (m.p. 183–184°), these two compounds account for 51·7% of doses of 0·5 g. A small amount of 4-hydroxyantipyrine (m.p. 183–184°) can also be isolated, and this occurs in the urine bound to glucuronic acid (Halberkann and Fretwurst, 1940). A glucuronide of unknown constitution was also isolated by Jaffe. The point of biological attack on the pyramidone molecule is obviously position 4, carrying the dimethylamino group. This group is first demethylated, both methyl groups being removed, and then the resulting $NH_2$ group undergoes acetylation ; finally the amino group is removed and replaced by a hydroxyl group. The methyl groups attached to positions 2 and 3 appear to be unattacked. That position 2 may be demethylated is suggested by the earlier work of Jaffe (1901). According to this worker, human and dog urine after the ingestion of pyramidone contains a red pigment which is known as rubazonic acid. The amount present in the urine corresponds to 1–3% of the ingested pyramidone. According to Jaffe, dog urine, after the continuous ingestion for long periods of 3–5 g. pyramidone per day, contains phenylmethylaminopyrazolone, which, after acidification and oxidation by atmospheric oxygen, is converted to rubazonic acid which separates as red flocks. The formula for rubazonic acid (m.p. 181°) is given by Knorr as follows :—



**rubazonic acid**

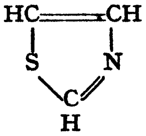


**antipyrylurea**

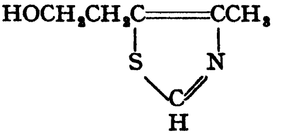
Jaffe also isolated from dog urine antipyrylurea (m.p. 245°) and the possibility exists that this compound, like rubazonic acid, is an artefact (see p. 105).

## THIAZOLE AND ITS DERIVATIVES

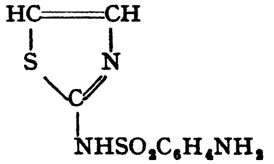
The thiazole ring occurs in two important compounds, namely, vitamin $B_1$ and the drug sulphathiazole. Information regarding the fate of the thiazole ring in the animal body is, however, entirely lacking. Parenteral administration of vitamin $B_1$ is said to cause a large increase in the excretion of urinary pyrimidine (Pollack, Ellenberg and Dolger, 1941), and from this one can conclude that it may be partly split *in vivo* to its thiazole and pyrimidine moieties, but there is no record of what happens to the thiazole fragment. *dl*-α-Amino-β-(4-methylthiazole-5)-propionic acid is fermented by yeast forming 4-methyl-5-(β-hydroxyethyl)-thiazole (the thiazole moiety of aneurin) and the *d*(-) form of the original amino acid. The possibility that this reaction might occur *in vivo* is considered by Harington and Moggridge (1939; 1940).



thiazole



4-methyl-5(β-hydroxyethyl)-thiazole

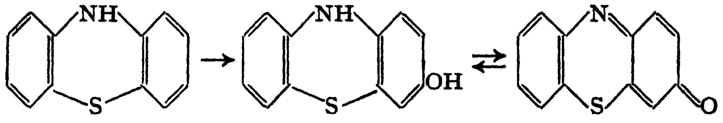


sulphathiazole

**2-Aminothiazole** (" Abadol ") is said to be superior to thiouracil in the treatment of thyrotoxicosis. It is rapidly destroyed by the body, its metabolites being unknown (Perrault and Bovet, 1946).

## THIAZINE DERIVATIVES

**Phenothiazine.** The metabolism of this important drug has been studied in rats, rabbits and humans (cf. DeEds, Eddy and Thomas, 1938; Collier, 1940). It is widely used in veterinary practice as an anthelminthic. Rats, rabbits and humans receiving the drug orally excrete a reddish urine and the colour becomes more intense when the urine is allowed to stand exposed to air. The red pigment can be extracted from acidified urine with a variety of solvents. This pigment is apparently an oxidation product of phenothiazine and is identical with thionol. The products excreted in the urine after oral administration of phenothiazine are the unchanged drug, leucothionol and its glucuronide (Benham, 1945), and thionol. Thionol and leucothionol apparently form a reversible oxidation-reduction system, thionol being responsible for the coloration of the urine.



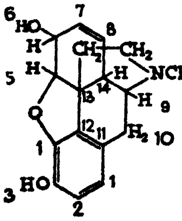
phenothiazine leucothionol (leucophenthiazone) thionol (phenthiazone)

Information regarding the fate of other important derivatives of thiazine such as methylene blue and Lauth's violet is entirely lacking.

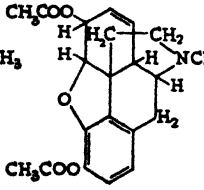
## MORPHINE AND RELATED COMPOUNDS

**Morphine.** Investigation of the fate of morphine in the animal body has for a long while attracted the attention of numerous workers, but it is only within the last few years that any tangible information regarding its metabolism has become available. The early work of Stolnikow (1884) indicated that in dogs morphine formed an ethereal sulphate; he also showed that the synthetic ethereal sulphate of morphine had different pharmacological properties from morphine: in fact, Oberst and Gross (1944) have recently shown that morphine ethereal sulphate does not produce euphoria or relieve pain as does morphine. A knowledge of the chemical changes which morphine undergoes in the body is important, for the recent work of Gross and Thompson (1940), while it does not explain addiction, suggests that habituation to morphine may lead to an alteration in its metabolism.

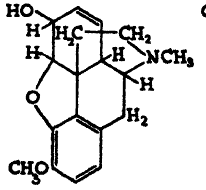
Chemically, morphine is a derivative of phenanthrene with two additional heterocyclic rings. It contains two hydroxyl groups, one occurring in a secondary alcohol group (position 6); the other is phenolic (position 3). Both these groups may undergo conjugation *in vivo*.



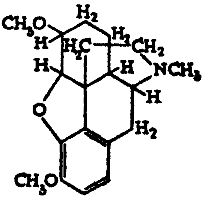
morphine



heroin



codeine



dihydrocodeine methyl ether

In dogs which have not acquired tolerance to morphine, as much as 80–92% of the dose irrespective of its size can be recovered in the urine. The greater proportion of the recovered material is in a combined form. On the other hand, dogs which have been tolerated to morphine for a year or more excrete only 50% (35–66%) of the dose, 30% being in the combined form. The decrease in morphine excretion in tolerant dogs is not in the free morphine excreted but in the combined form, and it appears that dogs tolerated to morphine can destroy it, whereas non-tolerated dogs have little ability to do so (Gross and Thompson, 1940). The products of the destruction of morphine in tolerant dogs are unknown.

As regards the exact nature of the conjugated forms of morphine excreted by the dog, we have but little information. According to Gross and Thompson, the dog excretes two conjugates, one of which is hydrolysed at *p*H 1 in 60 minutes at 100° and accounts for 30% of the combined morphine excreted by non-tolerated dogs. This conjugate is probably an ethereal sulphate in which the sulphuric acid group must be attached to the phenolic 3-hydroxyl group. The second combined form is more stable and is only hydrolysed to morphine by strong acid under steam pressure. The nature of this more stable conjugate is unknown. The ratio between the amounts of these two conjugates excreted is apparently related, in dogs, to the development of tolerance (Gross and Thompson, 1940).

Morphine is also conjugated when incubated *in vitro* with liver slices of the dog, cat, rat or guinea-pig, and the conjugate formed is probably a glucuronide for it is produced in the absence of the sulphate ion (Bernheim and Bernheim, 1945).

Conjugated morphine is also excreted by the human being (Oberst, 1940, 1941). In morphine addicts some 30% (11–55%) of varying doses of injected morphine is excreted in the urine, and of this 5% is free and 25% conjugated. 70% is unaccounted for and is presumably completely oxidised or eliminated as unidentified degradation products. That morphine is excreted as a conjugate is evident from the fact that in addicts there is a rise on the glucuronic acid output which is proportional to the dose of morphine ; this glucuronide is apparently the main conjugate of morphine in man (Oberst, 1941). The glucuronic acid residue could be attached to either the 3- or the 6-hydroxyl of the morphine molecule, but the exact nature of this glucuronide is at the moment undecided. When morphine is administered orally the amount excreted in the free state is less and that excreted combined is greater than when it is injected subcutaneously. At the same time, on changing from subcutaneous to oral administration, there is a slight increase in the dosage level necessary for physical dependence. Omitting any considerations of absorption from the alimentary canal, it may be that, on oral administration, more morphine is destroyed or conjugated (on absorption from the alimentary canal the drug passes into the portal system thence to the liver before reaching the general circulation) than during subcutaneous injection, thereby lessening the amount of drug available for effective physiological action (Oberst, 1940).

*α-Iso*morphine. Morphine and *α-iso*morphine are a stereoisomeric pair, the isomerism being due to the spatial arrangement of the H and OH groups attached to carbon atom 6 of the morphine molecule. In the human being it appears that more *α-iso*morphine is destroyed than morphine, for the amount of free and combined *iso*morphine excreted is less than in the case of morphine (see Table 29) (Oberst, 1941).

**Heroin** (3:6-Diacetylmorphine). In the human being heroin is deacetylated to morphine which is then excreted in the free and conjugated form. In morphine addicts some 50% of injected heroin (as the hydrochloride) is excreted as conjugated morphine and 7% as free morphine (Oberst, 1943). Heroin can apparently be deacetylated by rabbit and human sera (Wright, 1941), the acetyl group attached to the phenolic hydroxyl being more easily removed (Oberst, 1943).

TABLE 29

*The Conjugation of Morphine and its Derivatives (after Oberst,* 1941)

| Compound | Per cent of dose excreted in the urine | | Conjugated base / Free base |
|---|---|---|---|
| | as free base | as conjugated base | |
| Morphine | 6·2 | 32·8 | 5·4 |
| α-*Iso*morphine | 4·4 | 12·0 | 3·2 |
| 3:6-Diacetylmorphine (Heroin) | 5·8 | 43·5 | 8·7 |
| 3-Methylmorphine (Codeine) | 11·2 | 31·6 | 3·2 |
| Dihydrocodeine | 13·0 | 8·8 | 0·73 |
| Dihydro*iso*codeine | 18·3 | 18·4 | 1·0 |
| Dihydroheterocodeine | 8·1 | 37·8 | 5·5 |
| Dihydrocodeine methyl ether | 22·0 | 6·6 | 0·37 |

According to Oberst one-half as much heroin as morphine is needed to satisfy physical dependence in a morphine addict, and when morphine and heroin are administered in the ratio of 2:1 (i.e. equal satisfying power), 7% of each base is excreted as free morphine and 50% as conjugated morphine.

**Codeine** (3-methylmorphine). In codeine the phenolic hydroxyl group is methylated, but it is nevertheless excreted by man in conjugated form, for it has still a free hydroxyl group. The stereoisomeric pair dihydrocodeine and dihydro*iso*codeine, like morphine and α-*iso*morphine, show distinct differences in the extent to which they are conjugated in man. When both hydroxyl groups are blocked, as in the case of dihydrocodeine methyl ether, conjugation is prevented, and most of the injected methyl ether excreted is in the free state (see Table 29).

TABLE 30

*Biosynthetic Glycine Derivatives of Heterocyclic Carboxylic Acids*

| Compound | M.p. | Reference |
|---|---|---|
| Pyromucuric acid (furoylglycine) | 165° | Jaffe and Cohn (1887) |
| Furfuracryluric acid (furfuracryloylglycine) | 213–215° | ,, ,, ,, ,, |
| α-Pyridinuric acid | 164–165° | Cohn (1893) |
| Nicotinuric acid (β-pyridinuric acid) | 232° | — |
| α-Thiophenuric acid | 171–172° | Jaffe and Cohn (1888) |

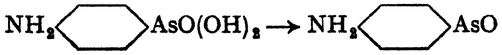
## *Chapter Thirteen*

# The Metabolism of Organic Compounds of Arsenic

THE use of organic compounds of arsenic for the treatment of protozoal infection is well known, but knowledge regarding the fate of such compounds in the animal is still unsatisfactory, despite the importance of such knowledge in explaining the therapeutic action of such drugs.

The majority of the drugs used are derivatives of phenylarsonic acid, $C_6H_5AsO(OH)_2$ or of arsenobenzene, $C_6H_5As{:}AsC_6H_5$, and they are characterised by a relatively low toxicity when compared with inorganic arsenic compounds. They are inactive against parasites *in vitro*, and *in vivo* they show a latent period during which there is no evidence of parasiticidal action. This latent period is followed by a gradual destruction of the parasites, for activation of the drugs involves to a great extent the participation of the host. These facts indicate that the active chemotherapeutic agents are not the drugs themselves, but compounds formed from them by the action of the body tissues.

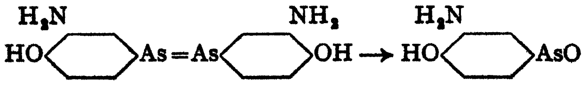
In the case of the quinquevalent arsenical compounds derived from phenylarsonic acid, Ehlich was of the opinion that they were reduced *in vivo* to the corresponding tervalent phenylarsenoxides. Thus, taking the case of atoxyl, *p*-aminophenylarsonic acid, it would be reduced *in vivo* to *p*-aminophenylarsenoxide.



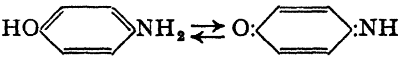
The arsenoxides are active against parasites *in vitro*, but there is *in vivo* an increase in toxicity for the host. The chemotherapeutic action of compounds such as atoxyl may therefore be explained by their gradual conversion *in vivo* into the more toxic but more parasiticidal arsenoxides, although other explanations must not be excluded.

A similar explanation has been put forward to explain the activity of compounds of the arsenobenzene type, for Voegtlin (1925) has obtained evidence which indicates that these compounds, e.g. arsphenamine and neoarsphenamine, may be oxidised *in vivo* to active arsenoxides :—

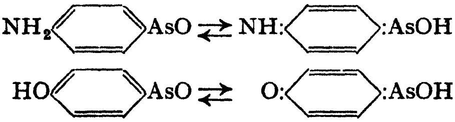
$$R.As{=}As.R \rightarrow R.AsO$$



Compounds of the arsenoxide type, e.g. mapharsen or 3-amino-4-hydroxyarsenoxide, appear to be more effective therapeutically than the arsphenamines (cf. Findlay, 1939*a*; Banks, 1944). Voegtlin (1925) suggests that the direct toxic action of the arsenoxides on the parasites is essentially due to an interference with cellular oxidation and reduction systems controlled by glutathione (Voegtlin and Smith, 1920), for glutathione and certain other compounds containing sulphydryl groups,[1] e.g. thioglycollic acid, afford some protection for the parasites against arsenoxides (Voegtlin, 1925). The work of Reiner and Leonard (1932) suggests that compounds of the arsphenamine type may form with serum proteins loose compounds which are active parasitotropic agents, for such adducts are present in the serum or plasma of animals treated with neoarsphenamine, and they can also be formed *in vitro* by mixing neoarsphenamine with serum or plasma. The possibility of compounds of the quinoneimine type has also been envisaged by Reiner and Leonard (1932*a*) as being formed from aromatic organic arsenicals. It is well-known that *p*-aminophenol undergoes autoxidation to iminoquinone.



*p*-Aminophenol is non-toxic to trypanosomes but iminoquinone and also *p*-benzoquinone are toxic. Similar oxidation compounds are possible with arsenoxides, thus :—



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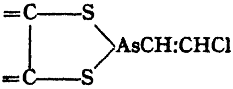
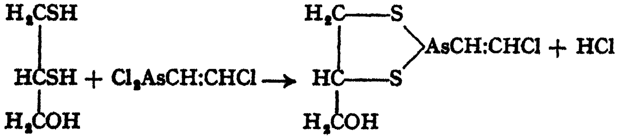
[1] Chlorovinyl arsine dichloride (lewisite), $CHCl{:}CHAsCl_2$, exerts its toxicity, according to Peters and his co-workers (see Peters, Stocken and Thompson, 1945), by inhibiting the " pyruvic oxidase " system, thereby interfering with the oxidation of carbohydrate at the pyruvate stage. An analogous state is found in thiamine deficiency. Lewisite, however, exerts its effect on the protein component of " Pyruvate oxidase ", the coenzyme withstanding the poison. It combines with —SH groups in the protein, probably forming ring compounds with " dithiol " proteins thus :—



There is no published information regarding any excretory products of lewisite.

The discovery that lewisite combines with —SH groups led Peters and his co-workers to the suggestion that simple dithiols might compete with " dithiol " proteins for lewisite and thereby protect the organism against arsenical burns. If the arsenical burn were not of too long standing, simple dithiols might also reverse the combination of lewisite with body proteins. A number of dithiols were therefore tested and the most effective was found to be 2:3-dimercaptopropanol (British anti-lewisite, BAL). This compound reacts with lewisite as follows :—



$$\begin{array}{l} H_2CSH \\ | \\ HCSH \\ | \\ H_2COH \end{array} + Cl_2AsCH{:}CHCl \longrightarrow \begin{array}{l} H_2C\text{—}S \\ | \qquad \rangle AsCH{:}CHCl \\ HC\text{—}S \\ | \\ H_2COH \end{array} + HCl$$

It is highly effective in preventing the toxic action of lewisite upon the pyruvate oxidase system of brain and in protecting human beings and animals against lewisite.

These highly important results show definitely that arsenicals of the lewisite type exert their toxic effects by combining with the sulphydryl groups of proteins in the animal.

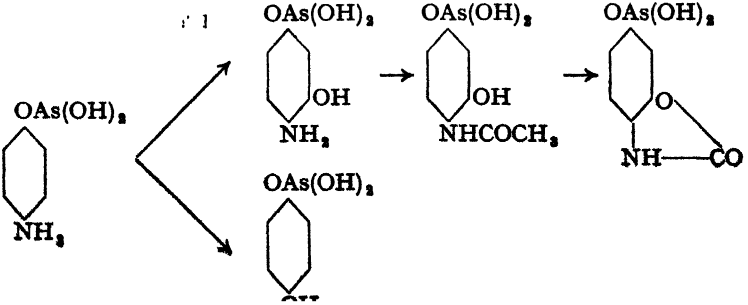
This toxicity of iminoquinone and of benzoquinone is inhibited by sodium thioglycollate (see Voegtlin above) which also protects trypanosomes against the arsenoxide. But the toxicity of benzoquinone is also inhibited by sodium thiosulphate and sulphite, whereas that of neoarsphenamine is inhibited only by thiol compounds. These facts indicate that the toxicity of neoarsphenamine is probably not due to the arsenoxide forming a quinoneimine type of compound.

The above facts make it clear that studies of the metabolism of organic arsenicals is highly important, but in the past most investigations have been confined to the estimation of inorganic and organic arsenic in blood, tissues and excreta and little attention has been paid to changes in the organic structures to which the arsenic atoms are attached.

**Dimethylarsonic acid (Cacodylic Acid).** It appears probable that this substance undergoes both oxidation and reduction in the animal body. According to Heffter (1901), when it is injected in small doses into man or the rabbit not more than about 14% can be accounted for by the various forms of arsenic excreted in the urine. The main substance excreted in the urine is unchanged cacodylic acid, although small amounts of arsenious and arsenic acids are also found. It appears probable that some tissues, particularly liver, stomach and intestine, can reduce cacodylic acid to cacodyl oxide (Heffter, 1901)

$$(CH_3)_2AsOOH \longrightarrow ((CH_3)_2As)_2O$$

***p*-Aminophenylarsonic Acid.** The monosodium salt of this acid is used under the name "*atoxyl*" and was one of the first organic arsenicals to be used in the treatment of experimental trypanosomiasis (Thomas, 1905). Its fate in the animal body was investigated by Nierenstein (1909), who injected it into a horse and examined the products excreted in the urine. Although a large proportion was broken down to inorganic arsenic, some was excreted unchanged and two organic arsenical metabolites were isolated. The latter compounds were *p*-hydroxyphenylarsonic acid and the arsonic acid of benzoxazolone (2-oxybenzoxazole-6-arsonic acid). The formation of the first compound is remarkable in that an aromatic amino group has been split off and replaced by OH. The second derivative is formed according to Nierenstein by *ortho* oxidation, followed by acetylation to form 4-acetamino-3-hydroxyphenylarsonic acid which is then converted to a benzoxazolone derivative—presumably by loss of methane, $CH_4$.



The formation of benzoxazalone derivatives has been discussed on page 144, and it is obvious that a reinvestigation of the formation of these compounds is urgently necessary.

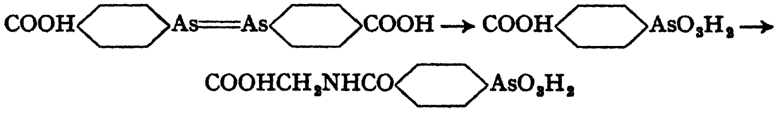
## ARSENO COMPOUNDS

Compounds containing the arseno group —As=As— are analogous with those containing the azo group, —N=N—. It is therefore interesting to compare the fate of these two groups in the animal body. Both are split *in vivo*, but whereas the azo group is reduced to two aromatic amino groups, possibly through an intermediary hydrazo compound, the arseno group is oxidised with the formation of arsonic acids, the arsenoxide being probably an intermediate stage in the oxidation :—

$$\mathrm{ArN{=}NAr'} \longrightarrow \mathrm{ArNHNHAr'} \longrightarrow \mathrm{ArNH_2} + \mathrm{Ar'NH_2}$$

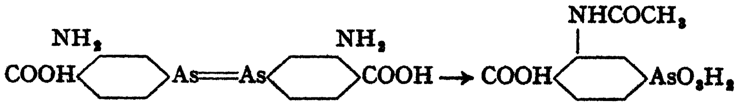
$$\mathrm{ArAs{=}AsAr'} \longrightarrow \mathrm{ArAsO} + \mathrm{Ar'AsO} \longrightarrow \mathrm{ArAsO(OH)_2} + \mathrm{Ar'AsO(OH)_2}$$

**4:4′-Arsenobenzoic acid**, when injected subcutaneously into the calf, is considerably degraded. Much of the arsenic is excreted in inorganic form and there is a large output of benzoic acid. The arseno link is split and the tervalent arsenic is oxidised to quinquevalent arsenic for *p*-carboxyphenylarsonic acid can be isolated from the urine; Sieberg (1916) is of the opinion that the arsonic acid is excreted conjugated with glycine, i.e. as *p*-arsonylhippuric acid.



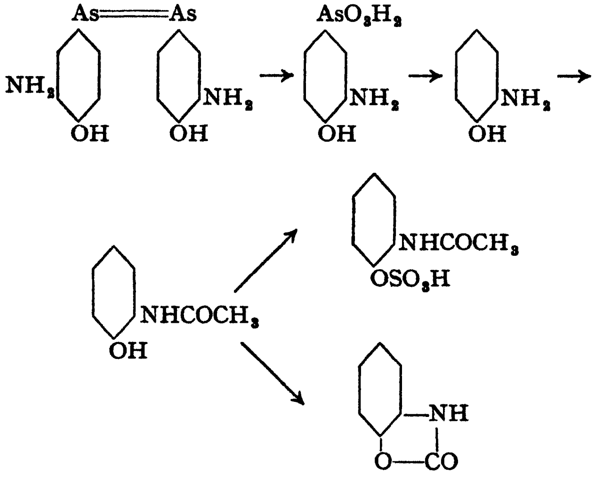
Calf urine after arsenobenzoic acid injections is strongly reducing, and Sieberg isolated from it the *p*-bromphenylhydrazone of glucuronic acid. It is probable that the glucuronic acid was not excreted in the free state as Sieberg suggested, but as the reducing ester glucuronide of benzoic acid and possibly of *p*-carboxyphenylarsonic acid.

The fate of **2:2′-diamino-4:4′-arsenobenzoic acid** in the calf is very similar to that of 4:4′-arsenobenzoic acid. The arseno link is split and much inorganic arsenic excreted. Sieberg isolated from the urine 3-acetamino-4-carboxyphenylarsonic acid, in which the original amino group has been acetylated *in vivo*; however, no glycine conjugate was detected.

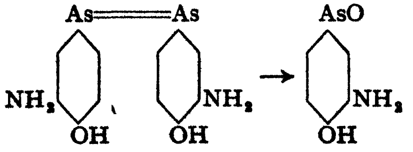


**Arsphenamine** (" 606 " or " salvarsan "; 1:1′-dihydroxy-2:2′-diamino-4:4′-arsenobenzene). This well-known drug, which was first used by Ehlich in the early part of this century for the treatment of syphilis, contains the arseno linkage —As=As—. The fate of this compound on injection into humans suffering from syphilis was investigated by Sieberg

(1916), who examined the products present in the urine of such patients. Some salvarsan is excreted unchanged and much is decomposed into inorganic arsenic. In addition, small amounts of *p*-aminophenol, *o*-acetaminophenylsulphate, benzoxazolone, 3-hydroxy-4-aminophenylarsonic acid and *p*-hydroxyphenylarsonic acid can be isolated from the urine. The arseno linkage is obviously split and oxidised as in the case of arsenobenzoic acid. The formation of *o*-aminophenol and its derivatives are therefore explicable, with the possibility that benzoxazolone is an artefact, as follows :—



The formation of *p*-hydroxyphenylarsonic acid, however, is more difficult to explain, for it involves the replacement of a nuclear $NH_2$ group by H. In the case of atoxyl it appears that the $—NH_2$ is replaced by —OH. The formation of *p*-aminophenol is even more difficult to explain, for it involves the loss of the arsonic acid group and a nuclear hydroxyl followed by an oxidation in another position. These results obviously require re-investigation, for Voegtlin (1925) has pointed out that Sieberg used methods of isolation for his compounds which were liable to give untrustworthy results and some of his products are likely to be artefacts. Rosenthal (1932) claims that one hour after injecting rats with either " salvarsan " or " neosalvarsan ", 3-amino-4-hydroxyphenylarsenoxide can be detected in the liver and kidneys.



The general impression which remains in the mind after reviewing the literature on the fate of organic arsenicals in the body is that the subject is in a rudimentary state and further research is needed.

## *Chapter Fourteen*

# Theoretical Considerations and Conclusions

IT will be by now clear that there is a considerable body of verified knowledge concerning detoxication mechanisms, and as would be expected, attempts have been made to reduce it to order. Underlying the factual information there must be some significant pattern, the perception of which is a major objective of research. In this chapter it is proposed to discuss and evaluate the various hypotheses which have been put forward regarding the biological significance of the detoxication mechanisms.

It must be admitted that interpretations worthy of the status of theories are lacking as yet, but certain hypotheses are at least worthy of careful scrutiny. The three which are dealt with below have the merits of trial hypotheses, but each is obviously inadequate.

It is proposed to designate the three hypotheses as (1) the chemical defence hypothesis, (2) the surface tension hypothesis and (3) the increased acidity hypothesis. The first is the most widely known, for it is the commonly accepted explanation of detoxication mechanisms. It dates back to 1876 when Baumann discovered the ethereal sulphates, and it was more fully developed by Sherwin and his school between 1920 and 1930. The other two hypotheses are more obscure and have received little attention.

## The Chemical Defence Hypothesis

In 1876 Baumann made the observation that the potassium salt of phenylsulphuric acid administered orally to animals was non-toxic. This was a discovery of fundamental importance for phenylsulphuric acid is a metabolite of the relatively toxic substance, phenol, and it appeared as if the body could protect itself against the toxic action of phenol by conjugating it with the sulphate ion. In 1910, however, Metchnikoff pointed out that, when injected, potassium phenylsulphate is definitely toxic and is far less innocuous than Baumann had indicated. Nevertheless, whether tested by injection or ingestion, the salt is definitely less toxic than phenol and the conversion of the latter to the conjugate is without doubt a true detoxication process (hypotoxic change).

Out of this discovery grew the concept that the changes undergone by foreign organic compounds in the body occurred *for the purpose of* reducing their toxicity and of thus protecting the animal from their poisonous effects. The notion that the animal may possess means of defending itself against chemical poisons is not unreasonable, for defence mechanisms of various kinds are not uncommon in nature. The view that detoxication processes are special defence mechanisms was fully developed by C. P. Sherwin and we may designate his conception " the chemical defence hypothesis ". He regarded detoxication as defence against toxic substances either formed *in vivo* or absorbed from the gastro-intestinal tract. Sherwin wrote :—

> It has been necessary for the body to call to its aid a chemical defence mechanism to guard against poison absorbed from the gastro-intestinal tract. After many generations the chemical defence mechanism has been so perfected that it is now quite able to cope with many of the foreign organic compounds . . .
>
> To attain these ends the body has at its command a series of chemical reactions— The first method of attacking a foreign molecule seems to be an attempt at *complete oxidation*. This, in the majority of cases, meets with at least partial success. . . . In some cases reduction is used as a precursor of oxidation, and in a few instances as an independent reaction. Should the foreign molecule be able to withstand these types of attack, or yield to them at most only partially, it is necessary for the body to have recourse to a synthetic type of reaction, combining the toxic substance with some radicle or molecule which it has at its disposal. In this way not only is the toxicity reduced but the resulting product is rendered more soluble.

TABLE 31

*The Solubilities in Water of Some Organic Compounds and Their Detoxication Products*

| Compound | Solubility mg./100 c.c. | Detoxication Product | Solubility mg./100 c.c. |
|---|---|---|---|
| Benzoic acid | 184 | Hippuric acid (in mammals) | 463 |
| | | Ornithuric acid (in birds) | 102 |
| Phenylacetic acid | 915 | Phenaceturic acid (in dogs) | 1,145 |
| | | Phenylacetylglutamine urea (man and chimpanzee) | 117 |
| *o*-Chlorophenylacetic acid | 311 | *o*-Chlorophenaceturic acid | 457 |
| *p*-Chlorophenylacetic acid | 222 | *p*-Chlorophenaceturic acid | 117 |
| *p*-Bromophenylacetic acid | 150 | *p*-Bromophenaceturic acid | 156 |
| Picric acid | 1,138 | Picramic acid | 65 |
| *m*-Aminobenzoic acid | 730 | m-Acetaminobenzoic acid | 100 |
| Sulphanilamide | 1,480 | $N^4$-Acetylsulphanilamide | 534 |

This statement postulates that the products of the detoxication mechanisms are (*a*) less toxic and (*b*) more soluble than the compound administered. This is a very attractive and simple explanation, but on examination of the facts as they are now known we find quite often that neither condition of the chemical defence hypothesis is satisfied. There are many instances of organic compounds which when introduced into the body are transformed into *more* toxic substances, and there are some cases where the product is *less* soluble at physiological *p*H values than the compound administered. Furthermore, a detoxication process may cause a foreign organic compound to become toxic in a different sense, for in some conjugation reactions, the administered compound combines with natural compounds which are essential to the body in the sense that they are required preformed and are not readily synthesised. There are therefore two ways in which a foreign compound may, through the so-called detoxication mechanisms, be harmful (toxic) to the organism ; (*a*) the biological conversion of the foreign compound, usually by oxidation or reduction, into compounds toxic in themselves, and (*b*) the conjugation of the foreign compound with an indispensable metabolite, thereby depriving the body of it for some essential process.

Let us consider briefly some examples of these. Nitro compounds are often transformed *in vivo* to toxic bodies by reduction; picramic acid, the biological reduction product of picric acid, is more toxic and less soluble than picric acid; 2:4:6-trinitrotoluene is transformed *in vivo* to dinitrohydroxylaminotoluenes which are highly toxic. Those nitro compounds which suffer little or no reduction *in vivo* are usually less toxic than those that are transformed, thus *ortho* substituted aromatic nitro compounds which undergo little reduction *in vivo* (e.g. *o*-nitrophenol and *o*-nitrobenzoic acid) are usually less toxic than the corresponding *para* compounds. In the case of many of the aromatic sulphonamides of the sulphanilamide type, the main detoxication process which they undergo is acetylation. The acetyl derivatives produced are often less soluble than their precursors and are frequently responsible for the kidney damage which sometimes accompanies treatment with sulphonamide drugs. Again, it has been shown that $N^4$-acetylsulphanilamide is more toxic than sulphanilamide. *p*-Bromophenylmercapturic acid is some ten times more toxic than its precursor bromobenzene, and when administered to dogs will cause haemoglobinuria (Callow and Hele, 1927). Many other examples of this type of detoxication mechanism can be quoted.

An instructive example of the second type of sequel to a detoxication process is the formation of mercapturic acids. The important amino acid cysteine is involved in this synthesis, and under some circumstances the baneful effect of certain hydrocarbons and their halogen derivatives is manifested by inhibition of growth, particularly in young animals. With limited supplies of cysteine, the animal may not obtain sufficient of this amino acid both for normal growth and well-being and for the conjugation process as well, and since the latter achieves priority the organism either ceases to grow or cysteine deficiency is manifested in other ways, e.g. cataract appears in rabbits receiving repeated doses of naphthalene (see p. 46). A similar state of affairs obtains in young rats which require glycine for normal growth and in which glycine synthesis is limited (Griffith, 1929). When these animals are given benzoic acid on a limited intake of dietary glycine they cease to grow. Survival and growth occur only when the diet provides a supply of glycine sufficient for the demands of both hippuric acid synthesis from benzoic acid and of maintenance and increase of tissue proteins. The probability of methyl deficiency being brought about by pyridine compounds is another example.

On the credit side, however, there are many cases in which the requirements of the chemical defence hypothesis appear to be satisfied. Whenever the ethereal sulphate and glucuronic acid conjugations occur there is a reduction in toxicity and greatly increased solubility at physiological *p*H values. The detoxication of hydrogen cyanide to thiocyanic acid is also a true detoxication process. It should be noted, however, that these cases are concerned with the detoxication of substances formed normally in small amounts in the body. Phenols, such as phenol and *p*-cresol which conjugate with sulphuric and glucuronic acids are normally formed in small amounts. In the sense that they deal with the small amounts of normally formed toxic bodies it is probable that true detoxication processes do occur, thus constituting a biochemical defence mechanism. It is also probable that these mechanisms can deal with moderately increased amounts of the natural toxic body and to some extent with foreign toxic substances which are chemically similar to naturally formed toxic compounds, but it is very unlikely that these mechanisms can invariably reduce the toxicity of chemical structures totally foreign to the body, and in these cases it would be mere chance if toxicity and solubility were simultaneously reduced by these mechanisms.

It is also probable that the ethereal sulphate and glucuronic acid conjugations possess true physiological functions in that they may be used for altering the physiological properties of a compound formed for a specific purpose by the body. Adrenaline, for example, is apparently inactivated by conjugation to an ethereal sulphate (see p. 76); progesterone is inactivated by reduction to pregnandiol and conjugation as pregnandiolglucuronide; oestrogenic hormones are also known to form inactive glucuronides and ethereal sulphates. The ethereal sulphate and glucuronic acid conjugations may therefore be mechanisms for altering the physiological properties of an endogenous compound rather than for detoxicating foreign compounds.

The possibility of rapid adaptation has also to be envisaged. Adaptation is implied in the quotation by Sherwin, given on page 241. There is little doubt that most of the changes which have been designated detoxication mechanisms are carried out by enzymes or systems of enzymes. It is therefore possible that enzymic adaptation, which is a common phenomenon in bacteria (for literature see Yudkin, 1939), may also take place in the animal body to enable it to deal with foreign organic compounds. Fishman (1940) has shown that when dogs or mice are given doses of menthol or borneol, there is an increase in the glucuronidase activity of certain tissues, particularly the liver, kidney and spleen. This increase in glucuronidase may be a response to the overloading of the tissues with substrates open to conversion by the enzyme into the corresponding glucuronides, or alternatively to overloading of the tissues with substrates for hydrolysis. It has similarly been observed that the power of the kidney to oxidise alcohol is increased by previous administration of alcohol (Lightbody and Kleinman, 1939). The enzymic aspect of detoxication mechanisms is, however, practically an untouched field.

## The Surface Tension Hypothesis

In 1917 Berczeller claimed that during a conjugation process a change in surface tension takes place so that the surface tension of a solution of the conjugated compound is nearer to that of water than the surface tension of a solution of the toxic or foreign body. Since there is an extraordinary large surface in the cells and other structures of the body, a compound which lowers the surface tension of water will tend to accumulate at these surfaces, and in consequence may attain such a concentration as to become toxic. If, however, the compound can form a conjugate such as a glucuronide or an ethereal sulphate in which the property of lowering the surface tension of water is not marked, there will be a lowering of toxicity, since the conjugated product will not tend to accumulate at surfaces in great concentration. Berczeller measured the surface tensions of solutions of a number of compounds and their detoxication products and obtained results which appeared to support this view. A saturated aqueous solution of hippuric acid has practically the same surface tension as water, whereas a saturated solution of benzoic acid has a much lower tension than water. Menthylglucuronide and menthol give similar results, a 0·125% solution of potassium menthylglucuronide being little different in surface tension from water. Phenol and three of its metabolic products, catechol, quinol, and phenylsulphuric acid, were also investigated by Berczeller. The lowering of the surface tension of water was greatest with phenol, less with catechol, still less with quinol, and least with phenylsulphuric acid. In fact, the surface tension of 1% phenylsulphuric acid is little different from that of water.

The possible relationship between surface tension and detoxication was further studied by Rose and Sherwin (1926), who recognised that, in the transference of toxic substances across semi-permeable membranes, factors which increase the concentration near the surfaces of such membranes are significant since they mobilise the toxic substances at points where they do most harm. If, however, detoxication gives rise to products the surface tensions of which are almost the same as that of pure water, they tend to be removed from susceptible surfaces and are also more easily excreted. Rose and Sherwin, however, criticised Berczeller's hypothesis because it stresses too strongly the quantitative aspect of toxicity and minimised the qualitative or specific aspect of toxicity.

Rose and Sherwin measured the surface tensions of solutions of a number of aromatic acids, and their metabolites, and their results are given in Table 32, which is quoted from their paper. In this table the percentage decrease in surface tension is noted, for as Rose and Sherwin point out, Berczeller's hypothesis can be stated in another way, i.e. the percentage lowering of the surface tension of water is greater for the substance fed than for the substance excreted. An examination of their data reveals that there are seventeen cases in favour of the surface tension hypothesis and six against it, whereas twenty-six substances are listed as excreted unchanged. From a consideration of solubilities and surface tensions, Rose and Sherwin attempted to decide whether the twenty-six substances excreted unchanged could be counted as being for or against the hypothesis. They argued that Berczeller's hypothesis implies that substances which are fairly soluble and give solutions possessing rather low surface tensions must undergo some change in the body whereby they lose their tendency to reduce surface tension. From this it follows that a compound with a very high ratio of solubility to surface tension can be expected to be detoxicated by the body. The lowest value for this ratio in compounds actually detoxicated and quoted in Table 32 is found in the cases of phenylpropionic and cinnamic acids. Rose and Sherwin, therefore, decided that substances which are excreted unchanged and in which the ratio is above this minimum value should count against Berczeller's hypothesis. Out of the twenty-six

TABLE 32

*Surface Tensions of Compounds and their Metabolites*

| Name of Compound | Concentration of saturated solution | Surface tension of saturated solution | Decrease in surface tension of water caused by saturation with the substance |
|---|---|---|---|
| | Mg./100 c.c. | Dynes | % |
| *Detoxication by Rabbit* | | | |
| Substance fed, benzoic acid | 184 | 62 | 17 |
| Excreted, hippuric acid | 463 | 71 | 3 |
| Substance fed, *o*-chlorobenzoic acid | 6 | 69 | 16 |
| Excreted, unchanged | | Same | |
| Substance fed, *o*-bromobenzoic acid | 4·5 | 73 | 5 |
| Excreted, unchanged | | Same | |
| Substance fed, *m*-bromobenzoic acid | 49 | 69 | 9 |
| Excreted, unchanged | | Same | |
| Substance fed, *o*-nitrobenzoic acid | 772 | 59 | 16 |
| Excreted, unchanged | | Same | |
| Substance fed, *m*-nitrobenzoic acid | 717 | 66 | 8 |
| Excreted, unchanged | | Same | |
| Substance fed, *m*-aminobenzoic acid | 730 | 69 | 8 |
| Excreted, *m*-acetylaminobenzoic acid | 100 | 69 | 13 |
| Substance fed, phenylacetic acid | 915 | 58 | 25 |
| Excreted, phenaceturic acid | 1,145 | 65 | 13 |
| Substance fed, *o*-chlorophenylacetic acid | 311 | 41 | 47 |
| Excreted, unchanged | | Same | |
| Substance fed, *p*-chlorophenylacetic acid | 222 | 55 | 20 |
| Excreted, unchanged | | Same | |
| Substance fed, *p*-bromophenylacetic acid | 150 | 59 | 25 |
| Excreted, unchanged | | Same | |
| Substance fed, *p*-hydroxyphenylacetic acid | 1,732 | 57 | 27 |
| Excreted, unchanged | | Same | |
| Substance fed, *o*-nitrophenylacetic acid | 360 | 72 | 10 |
| Excreted, unchanged | | Same | |
| Substance fed, *p*-nitrophenylacetic acid | 180 | 68 | 16 |
| Excreted, unchanged | | Same | |
| Substance fed, phenylpropionic acid | 36 | 53 | 31 |
| Excreted, hippuric acid | 463 | 71 | 3 |
| Substance fed, picric acid | 1,138 | 75 | 0 |
| Excreted, picramic acid | 65 | 75 | 0 |
| Substance fed, phenaceturic acid | 1,145 | 65 | 13 |
| Excreted, unchanged | | Same | |
| Substance fed, ornithuric acid | 102 | 69 | 10 |
| Excreted, unchanged | | Same | |
| Substance fed, cinnamic acid | 53 | 69 | 8 |
| Excreted, hippuric acid | 463 | 71 | 3 |

TABLE 32—*continued*

*Surface Tensions of Compounds and their Metabolites*

| Name of Compound | Concentration of saturated solution | Surface tension of saturated solution | Decrease in surface tension of water caused by saturation with the substance |
|---|---|---|---|
| | Mg./100 c.c. | Dynes | % |
| *Detoxication by Dog* | | | |
| Substance fed, *o*-chlorophenylacetic acid | 311 | 41 | 47 |
| Excreted, *o*-chlorophenaceturic acid | 457 | 65 | 17 |
| Substance fed, *p*-chlorophenylacetic acid | 222 | 55 | 20 |
| Excreted, *p*-chlorophenaceturic acid | 117 | 67 | 12 |
| Substance fed, *p*-bromophenylacetic acid | 150 | 59 | 25 |
| Excreted, *p*-bromophenaceturic acid | 156 | 66 | 14 |
| Substance fed, *m*-aminophenylacetic acid | 836 | 65 | 14 |
| Excreted, unchanged | | Same | |
| *Detoxication by Human Being* | | | |
| Substance fed, phenylacetic acid | 915 | 58 | 25 |
| Excreted, phenylacetylglutamine urea | 839 | 45 | 40 |
| Substance fed, *o*-chlorophenylacetic acid | 311 | 41 | 47 |
| Excreted, *o*-chlorophenaceturic acid | 457 | 65 | 17 |
| Substance fed, *p*-chlorophenylacetic acid | 222 | 55 | 20 |
| Excreted, *p*-chlorophenaceturic acid | 117 | 67 | 12 |
| Substance fed, *p*-bromophenylacetic acid | 150 | 59 | 25 |
| Excreted, *p*-bromophenaceturic acid | 156 | 66 | 14 |

substances excreted unchanged, twenty-two had ratios of solubility to surface tension above that for phenylpropionic acid and cinnamic acid and hence they were considered against the hypothesis. Thus, as a final result of their endeavours, Rose and Sherwin found twenty-one cases for and twenty-eight against the hypothesis. It should be pointed out, however, that many claims that compounds are excreted unchanged are based on inadequate data. Later more quantitative measurements have shown that metabolites excreted in small amounts can be often missed (cf. the nitrobenzoic acids, p. 137); the possibility of labile conjugates being excreted cannot be neglected. In our present state of knowledge it is doubtful whether compounds claimed to be excreted unchanged can be considered as counting for or against this hypothesis. Excluding compounds excreted unchanged, Rose and Sherwin's results show seventeen for the hypothesis and six against. In one case counted against the hypothesis, that of phenylacetic acid and its conjugates in man, phenylacetylglutamine urea, it has to be decided whether the conjugate is actually phenylacetylglutamine urea or phenylacetylglutamine, for combination of urea with conjugates often occurs during isolation procedures from urine.

As an hypothesis, the surface tension concept has not so far proved very serviceable, for it is too narrow in scope. It should be pointed out that the main postulate of the hypothesis is *a priori* a consequence of increase in molecular size. During a conjugation process there is produced a molecule larger than the one administered, and as the densities of most organic compounds vary over but a small range, it follows that the molecular volumes of conjugates are likely to be greater than those of the unconjugated compounds. Consequently, if we omit any considerations of polar orientation at surfaces, the number of conjugated molecules which will cover a given area of surface is less than that of unconjugated molecules. Therefore, the net surface tension effects due to the conjugated molecules will be less than those due to the unconjugated molecules.

### The Increased Acidity Hypothesis

On reviewing the nature of the compounds produced during conjugation processes in the body, it becomes obvious that in the majority of cases the excreted products are highly acidic compounds, e.g. glucuronic acids, arylsulphuric acids, hippuric acids, mercapturic acids, etc. Attention to this fact was drawn by Quick (1932*b*).

In Table 33 the dissociation constants of a number of compounds and their known metabolic products have been collected from various sources.

TABLE 33

*Dissociation Constants of Compounds and their Metabolites*

| Compound | Acid dissociation constant | Metabolic products | Acid dissociation constant |
|---|---|---|---|
| Benzoic acid | $6{\cdot}5 \times 10^{-5}$ | Hippuric acid | $2{\cdot}3 \times 10^{-4}$ |
| | | Benzoylglucuronide | $3{\cdot}0 \times 10^{-3}$ |
| Salicylic acid | $7{\cdot}41 \times 10^{-4}$ | Salicyluric acid | $2{\cdot}29 \times 10^{-4}$ |
| | | Gentisic acid | $4{\cdot}79 \times 10^{-4}$ |
| Phenylacetic acid | $5{\cdot}6 \times 10^{-5}$ | Phenaceturic acid | $2{\cdot}0 \times 10^{-4}$ |
| Veratric acid | $3{\cdot}6 \times 10^{-5}$ | Veratroylglucuronide | $0{\cdot}95 \times 10^{-3}$ [1] |
| Vanillin | $4{\cdot}7 \times 10^{-6}$ | Vanillic acid | $2{\cdot}9 \times 10^{-5}$ |
| *p*-Nitrobenzoic acid | $3{\cdot}9 \times 10^{-4}$ | *p*-Aminobenzoic acid | $1{\cdot}15 \times 10^{-5}$ |
| *p*-Aminobenzoic acid | $1{\cdot}15 \times 10^{-5}$ | *p*-Acetaminobenzoic acid | $5{\cdot}1 \times 10^{-5}$ |
| *l*-Menthol | — | *l*-Menthylglucuronide | *ca.* $1{\cdot}0 \times 10^{-3}$ |
| Phenol | $1{\cdot}3 \times 10^{-10}$ | Catechol | $3{\cdot}3 \times 10^{-10}$ |
| | | Quinol | $1{\cdot}1 \times 10^{-10}$ |
| | | Phenylsulphuric acid | A strong [2] acid |
| *p*-Nitrophenol | $6{\cdot}5 \times 10^{-5}$ | *p*-Nitrophenylsulphuric acid | A strong [2] acid |

[1] Unpublished determination by Dr. G. T. Mills.

[2] Burkhardt *et al.* (1936) state "potassium phenylsulphate and potassium nitrophenylsulphate were shown to be salts of strong acids, for, with HCl, they had no buffering action detectable by indicators . . . ; potassium sulphate in similar concentration showed marked buffering action".

It can be seen from Table 33 that in the majority of cases quoted the metabolic products are more acidic than their precursors. Notable exceptions are salicylic and *p*-nitrobenzoic acids. Quick states that it has to be decided whether the production of " strong " acids by the mechanisms of conjugation is merely incidental or whether it constitutes a fundamental factor in the detoxication and excretion of a foreign substance. As a test case Quick gives the example of benzoic acid. This practically non-toxic[1] substance is converted *in vivo* to the more acidic hippuric and benzoylglucuronic acids, hence Quick concludes " that conjugation may not primarily be a detoxication mechanism in the sense of rendering a toxic group inert, but a means of changing a weak acid which the body cannot excrete to a strong acid which it can eliminate ". The latter part of this statement, however, may not be strictly correct, since there is little doubt that weak acids can be excreted by the organism. The difference between the excretion of a weak acid and a strong one, if one disregards any specific physiological property a given weak acid may possess, lies probably in the time factor, since the body is likely to eliminate a strong acid much more rapidly than a weak one. Quick adduces further evidence in support of his ideas from the fact that relatively strong acids such as mandelic acid ($K = 4{\cdot}3 \times 10^{-4}$) and *o*-nitrobenzoic acid ($K = 6{\cdot}2 \times 10^{-3}$) are excreted unchanged. Here, again, the question arises whether these acids are actually eliminated quantitatively without change. It is known that *o*-nitrobenzoic acid does undergo reduction to some extent in the rat (see p. 137), whereas mandelic acid may be partly converted to benzoylformic acid (see p. 114). This hypothesis is, like the surface tension hypothesis, very narrow in scope, and there are many cases where it does not fit the facts. It is further limited by the difficulty of classifying all acids as strong or weak.

## Conclusions

The three hypotheses outlined above are inadequate as explanations of the changes involved during the metabolism of foreign organic compounds in the animal body. All are too narrow in scope, for each one is concerned with the alteration in only one or two biologically significant properties of a compound during its metabolism. Thus the chemical defence hypothesis, which is the most adequate of the three, is concerned with toxicity and solubility, the surface tension hypothesis with the relative values of surface tension and the increased acidity hypothesis with acid strength. In many instances compounds have been found the metabolism of which fit these hypotheses, whereas on the other hand many examples can be quoted which do not. It may, therefore, be pertinent to enquire whether a unitary theory of " detoxication mechanisms " is not something of a " will o' the wisp " at the present time.

Any sound theory of detoxication should answer the following questions :—

(1) Why are some compounds converted into more toxic compounds *in vivo*, whereas others undergo a true detoxication ?

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[1] Hippuric has L.D.50 = 4·15 g./kg. (Mattis, Beyer, McKinney and Patch, 1945), and is about a half as toxic as benzoic acid.

(2) Why are some compounds converted into more soluble products and others into less soluble products?

(3) Why does the same compound undergo changes which differ qualitatively and quantitatively in different species of animals?

(4) Why may a compound undergo a number of different changes simultaneously or a series of changes involving a chain of reactions, and what determines the amount of each metabolite formed?

It should also be possible by means of a sound theory to predict the fate of a given compound. To the writer it seems unlikely that the above questions can be covered by a unitary hypothesis.

From the standpoint of metabolism, toxic substances may be divided into two main groups: (*a*) those formed normally in the intestinal tract through the action of bacteria and to which the animal is accustomed and those ingested in the natural food of the animal, and (*b*) compounds *totally* foreign to the body, i.e. most synthetic organic compounds.

Let us now examine these two types of compounds in relation to those properties which are said to change according to the three hypotheses discussed above. It is to be conceded that the lowering of toxicity, the increase in solubility, the approach of surface tension to that of water and the increase in acidity are to the advantage of the organism.

Examples of toxic bodies which are formed naturally are phenolic substances such as phenol, *p*-cresol and indoxyl, aromatic acids such as benzoic and phenylacetic acids (although these two acids are relatively non-toxic), and perhaps hydrogen cyanide (in herbivores). The main metabolites of phenol are phenylsulphuric and phenylglucuronic acids. Phenylsulphuric acid is much less toxic and much more soluble than phenol; the surface tension of its 1% aqueous solution is the same as water, whereas that of phenol is much lower; phenol is a very weak acid, whereas phenylsulphuric acid is strongly acidic. In fact, the conversion of phenol to phenylsulphuric acid fits the requirements of all three hypotheses. Similarly phenylglucuronic acid is less toxic, more soluble and much more acidic than phenol, although nothing is known regarding its surface tension properties. Thiocyanic acid[1] is less toxic and more acidic than hydrogen cyanide. Hippuric acid is less toxic, more soluble, more acidic and the surface tension of its saturated aqueous solution is nearer to that of pure water than benzoic acid. A similar state of affairs is to be found with phenylacetic and phenylaceturic acids and with benzoic and benzoylglucuronic acids. At this point it should be pointed out that in the case of benzoic acid and its metabolite in the fowl, ornithuric acid, the latter substance is somewhat less soluble than benzoic acid, although the surface tension of its saturated solution is nearer to that of water than benzoic acid (see Table 29) but here one has to bear in mind that the fowl normally excretes such insoluble substances as uric acid, a condition which is related to its cleidoic egg-habit (see Baldwin, 1937).

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[1] The dissociation constant of HCN at 18° $= 1 \cdot 32 \times 10^{-9}$. No values have been quoted for thiocyanic acid, but it can be concluded from the facts that thiocyanates are stable salts whereas cyanides are comparatively unstable and that, from electrical conductivity measurements, $\Lambda_{0 \cdot 03}$ for HCN $< 0 \cdot 5$ and for HCNS $= 358$, that HCNS is a much stronger acid than HCN.

It is probable, therefore, that qualitatively all the advantageous changes postulated by the three hypotheses actually occur during the metabolism of natural toxic bodies to which the animal is accustomed, and the views expressed by all three hypotheses should be combined to explain the metabolism of compounds of this type. There is, however, a quantitative aspect. If the naturally formed toxic body is introduced into the animal in quantities larger than those found normally, the animal is poisoned, for phenol, *p*-cresol and cyanide poisoning are well known. The natural detoxication mechanisms are therefore chemical defence mechanisms of limited capabilities, not designed for large quantities of toxic bodies but only for the usually small amounts formed during normal metabolic processes. It is possible that in some species specific detoxication mechanisms are specially developed. Here it may be said that the chemical defence hypothesis is justified.

Consider now the changes involved during the metabolism of compounds totally foreign to the body. In those cases where the foreign compound possesses a chemical similarity to the normally formed toxic bodies, qualitatively similar changes take place. Totally foreign phenols, for example, form ethereal sulphates and glucuronides just as do the naturally formed phenols, and true detoxication occurs. However, from a quantitative aspect the extent of these conjugations may be entirely different from that occurring with the naturally formed phenols (see p. 72 and Fig. 1), the deciding factor being the influence of other groups present in addition to the phenolic group (Williams, 1938). In the case of the rabbit, salicylic acid is not conjugated as an ethereal sulphate, whereas *o*-aminophenol conjugates with sulphate to a greater extent than phenol. It appears, therefore, that it is sheer chance as far as the organism is concerned whether a compound can be detoxicated in the literal sense of the word, if it is totally foreign.

When considering the fate of an organic compound in the body, it is obvious that the primary entities concerned are the compound and its environment, the animal body. It is the interaction between these two entities which must be considered. When a foreign organic compound is introduced into the body it comes in contact with certain biochemical systems, mainly enzymic, which carry out the oxidations, reductions, hydrolyses, syntheses, etc., of the body in its normal state. The changes undergone by foreign organic compounds are the result of the interaction between these compounds and the normally occurring biochemical systems of the body. One can imagine these systems as the reagents with which the foreign compounds are treated when they enter the tissues of the organism. The products formed in these reactions may or may not be less toxic than the original compound. Alteration in toxicity, solubility, acidity and surface tension in favour of the organism may not occur.

The lock and key analogy may be useful to clarify the argument.

If the foreign organic compound happens to be an α-amino acid, it may be a " key " which fits the α-oxidative deamination " lock ", and the amino acid acetylation " lock ". It is to be expected, therefore, that the foreign amino acid will be partly or wholly deaminised and/or acetylated, the extent of either process depending on how well the " key " fits the " lock ". Benzene obviously is a " key " which fits some oxidising system in the liver, the nature of which is at the moment obscure ; the product of the oxidation is phenol which now fit the ethereal sulphate and glucuronic acid conjugation systems. Propylene glycol fits an oxidising system, possibly the alcohol dehydrogenase system, which partly converts it to lactic acid ; the latter now falls into its normal metabolic rôle, being partly converted into glycogen. Thus the completely foreign substance propylene glycol gives rise to the very natural substance, glycogen.

It appears to the writer that prediction as to the nature of the metabolites of a foreign organic compound must rest upon (*a*) analogies from the experience gained by studying closely related compounds and (*b*) knowledge concerning the degree of specificity of known (and unknown) enzyme systems of the body. Such enzyme systems may vary from species to species both qualitatively and quantitatively, and this variation may contain the key to an explanation of why a compound follows different metabolic routes in different species of animals.

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